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Potassium Use Efficiency in Barley (*Hordeum vulgare* L.) Genotypes Under Salt Stress Conditions

By

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Abstract

The growing global human population and the expansion of agriculture into less productive areas result in an increased demand for fertilisers. At the same time, sustainability of agricultural practices and increasing cost of fertilizers both call for reduced input and a use of genotypes with increased fertiliser use efficiency. This trend is applicable to all major fertilisers including potassium (K^+). Potassium is an essential macronutrient for plants and plays a crucial role in growth, development, yield, quality, quantity and stress resistance of all plant crops. The use of K^+-efficient genotypes along with optimised soil fertilisation would be important to develop optimal nutrient management strategies for sustainable farming systems. High potassium-use efficiency (KUE) genotypes can tolerate nutrient deficiency because they have adapted physiological mechanisms that enable access to adequate levels of specific nutrients (uptake efficiency) and use that nutrient effectively (utilisation efficiency).

The issue of KUE is further exacerbated by the fact that K^+ biological availability is severely affected by hostile environmental conditions. One of them is soil salinity. Salinity affects 10% of the earth's land surface and around 50% of all irrigated land, leading to lost agricultural production in excess of $27.3Bln per annum. At the same time, K^+ nutrition, both directly and indirectly, affects plant resistance to a broad range of abiotic and biotic stress. The causal relationship between KUE and salinity stress tolerance, nonetheless, remains elusive. In this project, we used a wide range of barley (*Hordeum vulgare* L.) genotypes contrasting in salinity stress tolerance to understand mechanisms conferring KUE and understand how these are affected by the presence of high NaCl concentrations in the rhizosphere.

Thirty barley genotypes, originating from Australia, China, USA and Japan were evaluated in a randomized block design with four levels of K^+ (0.002 mM, 0.2 mM, 2 mM, and 20 mM) in three replicates, for phenotypic and physiological variation in K^+ efficiency in shoot growth and grain yield. A subset of genotypes with contrasting KUE identified in the first experiment (highly efficient varieties YF374, Skiff and Yan 89110; and genotypes with low KUE - Dayton, Dysyh and Franklin) was used in the second experiment. Here, plants were grown at two potassium levels (low, 0.002 mM; and high, 20 mM) under two sodium levels (no salt and in the presence of 300 mM NaCl) for five weeks.

The results showed that the availability of K^+ in the soil had a major effect on yield components, i.e. spike number, grain number and grain weight, in most of the studied genotypes and the increase in grain weight in the response to K^+ application was correlated with an increase in

numbers of spikes and grains. Although an increase in K^+ supply led to an increase in plant height in all genotypes, the extent of response differed significantly between genotypes. K^+ availability did not increase tiller number in barley and 0.02 mM K^+ would be the threshold of deficiency for tiller number for most genotypes. The positive effect of K^+ on yield components, plant height and plant dry weight might be due to the role of K^+ in activating protein synthesis and improved enzymatic and photosynthetic activity, which shifts assimilates to sink and produce more grains with heavier weight.

K^+ supplementation caused only a small increase in plant dry shoot, which indicated that shoot dry weight alone cannot be used as a suitable selection criterion to detect tolerance of K^+ deficiency. It might be due to the fact that K^+ utilization influenced the translocation of dry assimilates into grains and caused a reduction in dry biomass. This variable response to low K^+ exhibited by these genotypes could be because of the difference in their ability to absorb K^+ and translocate it by K^+ transporter channels in the high and low-affinity uptake systems.

K^+ availability led to an increase in K^+ uptake and leaf K^+ content. Elevated leaf K^+ content correlated with higher xylem K^+ content, pointing out at essentiality of xylem K^+ loading as a key trait conferring KUE. The leaf Na^+ has an opposite trend to leaf K^+. The high performing plants showed the lowest leaf Na^+ and the lowest yielding plants showed high leaf Na^+. This indicates that plants that are not capable of accumulating enough K^+ instead rely on Na^+ to partially replace Na^+ with K^+ for opening and closing of stomata, which helps to regulate internal water balance.

The result of the second experiment showed that the application of K^+ alleviates the adverse effect of salinity, significantly improved the yield, and yield components, fresh and dry weight. Under salinity stress, genotypes Dayton, Dysyh and Franklin did not produce any grain regardless of the amount of K^+ applied (except Dysyh that produced 0.02 g/plant in high K^+), but genotypes with high KUE were doing well under saline conditions. Salinity stress caused an increase in leaf osmolality and a decrease in stomatal conductance. Leaf chlorophyll content (SPAD readings) was adversely affected by salinity; increased K^+ availability reverted this effect.

The overall outcomes of this work are three-fold. First, varieties with high KUE (Gebeina, Skiff, YF374, and Flagship) may be recommended to growers. Second, contrasting varieties selected in phenotyping experiments can be used to create DH lines to understand the genetic basis of KUE. Finally, this project provided further insights into mechanisms underlying KUE

and plant responses to salinity. While a competitive interaction between the K^+ and Na^+ and their transport into plant parts exist, Na^+ uptake showed a strong dependence of KUE, with K^+-efficient cultivars being more responsive to reduce Na^+ uptake under K^+ supply along with greater increases in K^+ uptake compared with K^+ inefficient cultivars. In the current study, application of salt stress promoted the uptake of Na^+ in plant leaves and xylem cells; however, the application of K^+ reduced its uptake in plants and promoted the accumulation of K^+ contents in plant leaves and xylem cells. This study also confirmed that application of supplemental K^+ could significantly ameliorate the toxicity of Na^+ to promote plant growth in soil-affected soils.

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Statement of Co-Authorship

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Chapter 1 Introduction

In the near future, food production will have to increase drastically. Not only is there an urgent need to develop crop plants with higher yields, but this has to be achieved with a minimal rise in use of increasingly scarce resources such as fertiliser and water. The availability of the three top elements potassium (K^+), nitrogen (N) and phosphorous (P) strongly determine the crop yield (Dreyer 2014). K^+ is the most abundant element and plays a crucial role in growth, development, yield, quality, quantity and stress resistance of all plant crops (Schroeder 1978; Wedepohl 1995; Zörb et al. 2014). To satisfy the plant's K^+ demand, with minimum use of fertilizer, a better understanding of the roles of K^+ in plants is required. The aim of this research is to identify plants with improved K^+ use efficiency (KUE) to help achieve this goal.

The global population is generally projected to reach 9.3 billion by 2050 (Lee 2011; United States Census Bureau 2012). This growth will occur mostly in areas where people currently suffer from nutritional disorders (Byrnes & Bumb 1998). Worldwide 30% of children are underweight, an indicator of child malnutrition, particularly for those under five years of age (Bank 2016a; Barbara et al. 2015; Pinstrup-Andersen 1999). An increase in food production is needed to address such current health issues, as well as to feed the expected increase in population, which will itself put serious pressure on existing agricultural land (Alexandratos 1995; Lee 2011; Nieves-Cordones et al. 2016). For example, global food production is predicted to increase by 50% by 2050 to meet the doubling demand with this increase in the population, however, the area of available land will only increase by 10% (Bijl et al. 2017; Evenson 1999; Ray et al. 2013; Tillman et al. 2011). The expansion of productive land is limited by environmental factors, such as salinity, waterlogging, frost and soil acidity (Bee 2014; Evenson 1999).

Significant increases in food prices and the continuing economic crises, particularly in the developing world, are impacting global malnutrition and the overall nutrition status of the human population. Improved crop production, better agricultural practices and adequate nutrition can only be achieved with the addition of mineral nutrients. Low fertiliser application in agricultural systems leads to the quick reduction of K^+ in the soil, resulting in a variety of negative effects in the utilisation of functional N and P fertiliser (Cakmak 2002). Widespread K^+ fertilisation (in the form of potash) for crop production is required, leading to high demand for K^+. Global K^+ fertiliser use is currently around 35 Mt/year and growing by about 3% annually (Food and Agriculture Organization of the United Nations (FAO) 2008). Large

amounts of K^+ fertiliser is applied in intensive agricultural systems to maximise crop production even though K^+ concentrations in most agricultural soils are high. However, as in most soil solutions K^+ is low (typically 10–100 µM), there is often insufficient K^+ available to support maximal crop growth. In extensive agricultural systems, the K^+ demand of high-yielding crops is rarely met. There is a need to develop highly nutrient-efficient plants because of a growing interest in low input agriculture, reducing fertiliser cost, and to achieve sustainability and high quality (Fageria et al., 2009; White 2013; Fageria and Moreira 2015).

Potassium fertilisers are used in agriculture to maximise production and are important to optimise the efficiency of crop plants under pressure from a lack of K^+ availability in the soil which improves KUE under stress conditions (Baligar et al. 2001; Pettigrew 2008; Rengel & Damon 2008; Szczerba et al. 2009; Trehan 2005). With the progressive intensification of agriculture and the introduction of high yielding varieties of crops, soils are becoming depleted in K^+ reserves at a faster rate. Consequently, K^+ deficiency has become one of the major constraints to crop production (Cakmak 2005). It is important to consider whether current K^+ management recommendations are sufficient to account for future changes. Recent research suggests instead of remaining constant, K^+ requirements vary with crop management factors and yield. In addition, the actual crop response to K^+ may not be accurately represented in commonly used soil tests. There is evidence to suggest genotypic differences affect responses to soil and fertiliser K^+, and traits such as stalk strength and the quality of the grain should be considered when managing K^+. Consequently, K^+ management recommendations should be more robust and accommodate factors such as differing crops, cropping systems, crop management technologies, soil conditions, and climate-driven yield potential (Dobermann 2001).

Stressors that affect the growth and yield of crops include: salinity, drought, waterlogging and low temperature (abiotic factors); bacterial and fungal diseases, insects and other pests (biotic factors), all of which can significantly reduce crop yield (Brouder & Volenec 2008). Therefore, agricultural systems face major challenges to become more resource-efficient to enhance crop yields and to make plant development and yield formation stable under biotic and abiotic stress conditions (Reynolds et al. 2011). High priority should be given to reduce the effects of abiotic stresses for sustainable food security through employing breeding and biotechnological approaches and improving soil fertility and productivity through ensured available minerals. Plants uses as wide range of adaptive mechanism to ensure survival and to maintain crop productivity under various biotic and abiotic stresses. The mineral nutrient status of plants play

an important role in improving environmental stress resistance in plants. For example, K^+ nutrition directly and indirectly improves plant resistance to both abiotic and biotic stress (Min et al. 2013; Zorb et al. 2014). K^+ is essential for many physiological processes such as photosynthesis, the activation of enzymes, translocation of photosynthates and to reduce the excess uptake of toxic ions like Na^+ in saline conditions (Mengel & Kirkby, 2001).

Soil salinity is a major abiotic stress intimidating crop production globally. It affects 10% of the earth's land surface and around 50% of all irrigated land, leading to lost agricultural production of US $12 billion per annum (Flowers et al. 2010; Ruan et al. 2010). Other factors aggravating the salinity problem include the loss of agricultural land to municipal land, the increasing contest between crops and energy plants, and the imperative need to increase food production by 70% by 2050 (Shabala S 2013). Soil salinity generally results due to accumulation of excess NaCl and reduces crop production through inducing ion toxicity and osmotic stress in plants (Acosta-Motos et al. 2017). Similar to other environmental stresses, salinity impairs photosynthesis, induce water deficiency, oxidative stress, ion toxicity, and the deficiency of minerals especially K^+ (Chakraborty et al. 2016; Zhu 2001).

Developing more efficient crop genotypes is an important challenge, particularly in terms of nutrient efficiency. The constant need for better-performing crops arises from demand for low-input agriculture, to minimise fertiliser costs and to achieve sustainability. The more efficient genotypes have specific physiological mechanisms or traits facilitating uptake efficiency and utilisation efficiency (Rengel & Damon 2008; Sattelmacher et al. 1994). The use of K^+-efficient genotypes along with optimised soil fertilisation would be important to a nutrient management strategy for sustainable farming systems (Rengel & Damon 2008). Impaired K^+ nutrition and Na^+ accumulation is a major characteristic of salt affected plants. The K^+ : Na^+ ratio in plants is a useful attribute for evaluating salt tolerance in plant species and genotypes. Selection of genotypes having a high K^+ : Na^+ ratio is an intervention technique to improve plant growth under saline conditions (Haq et al. 2014). Salt-tolerant genotypes responded to salinity through promoting an antioxidative defence to detoxify ROS (He et al. 2015; Shabala & Pottosin 2014; Min et al. 2013; Zhu 2001). Plant species and genotypes may differ in KUE. Genotypes having high KUE can tolerate deficiency of K^+ due to physiological mechanism which enable genotypes to access and uptake K^+ and use it efficiently (Rengel 2005; Rengel & Marschner 2005).

Even though the tolerance mechanisms of barley genotypes are widely studied, there is little information on the agronomy and physiology of KUE of barley genotypes under normal and

saline conditions. This dissertation consists of six chapters, two major environmental constraints (K^+ deficiency and salt-stress), with their global picture in food security and agriculture productivity reviewed. Furthermore, the most important key constraints imposed by each target stress on plant growth, physiology and yield were described along with their tolerance mechanism. The prospective adaptive/tolerance mechanisms related to salt-stress is discussed in detail.

Chapter 2: Literature review.

Chapter 3: Effects of K^+ availability on growth and development of glasshouse grown barley cultivars.

Chapter 4: study conducted under controlled conditions to evaluate the physiological basis of variation in KUE in a range of barley genotypes.

Chapter 5: Study conducted under controlled conditions to evaluate the effect salinity has on physiological and agronomical variation in KUE in barley.

Chapter 6: General discussion.

Chapter 2 Literature Review

2.1 K^+ in plants

K^+ is the most important cationic nutrient in barley (*Hordeum vulgare* L.) and other higher plants and plays an important role in the biochemical and biophysical processes of the plant, both at the cellular and whole-plant levels. Of particular importance are metabolism, turgor cell and water balance maintenance (Leigh & Wyn Jones 1984; Naseem et al. 2014; Pettigrew 2008; Sparks & Huang 1985).

The cumulative effect of the diverse functions of K^+ means most plants have relatively high levels of K^+, with cellular concentrations typically >100 mM. The cytosol, vacuole and organelles contain 50–200mM of K^+ (Leigh & Wyn Jones 1984). In some specialised structures such as guard cells, the K^+ concentration may even reach 500mM (Outlaw 1983) and K^+ can make up to 10% of plant dry weight (Leigh & Wyn Jones 1984; Tisdale et al. 1993; Watanabe et al. 2007) and at least 2% of plant tissue (Bergmann 1992). K^+ concentrations vary depending on crop species and fertiliser input, with the concentration ranging between 0.4 and 4.3% (Askegaard et al. 2004). Nevertheless, the K^+ levels can vary 5 to 10–fold in different species that have similar growth rates (Ramirez et al. 2005) or in the same species depending on K^+ supply (Szczerba et al. 2006; Walker et al. 1996).

K^+ has the ability to balance the charge of soluble (e.g. organic acid anions and inorganic anions) and insoluble anions, thus stabilising the pH between 7 and 8 in the cytoplasm, which is optimum for most enzyme reactions (Marschner 2012). K^+ functions in cell extension and other turgor-driven processes. At the cellular level K^+ catalyses many metabolic reactions, and most plant enzymes such as pyruvate, kinase and starch synthetase need K^+ to activate (Leigh & Wyn Jones 1984; Walker et al. 1996). K^+ is essential for the formation and maintenance of the membrane potentials and in the stroma for photosynthesis (Marschner 1995). Additionally, K^+ is indispensable as a counter-ion for cytoplasmic polyanions with a low charge–mass ratio (Wu et al. 2013; Leigh & Wyn Jones 1984; Walker et al. 1996).

In addition to these cellular functions, K^+ supply impacts on many whole plant processes including the distribution and efficacy of many other nutrients. For example, K^+ is needed in the xylem as a counter-ion for the long-distance transport of nitrate and other anions; and

nitrogen recovery is greatly increased in the presence of K^+, due to the role of K^+ in activating enzymes that function in ammonium assimilation (Hagin et al. 1990). The main biophysical role of K^+ is to provide turgor cell and water balance. As K^+ is the most abundant cation, it plays an important role in cell expansion, plant growth and plant movement (Moran et al. 1988). Consequently, increased K^+ nutrition has a large positive effect on drought resistance, water use efficiency, and yield quality. For example, there is a positive relationship between increased K^+ and the process of photosynthesis. K^+ determines the number and size of leaves, resulting in an increase of photosynthetic source material per unit leaf area, which in turn increases the availability of photosynthetic assimilates for plant growth (Leigh & Wyn Jones 1984; Maathuis & Sanders 1996; Naseem et al. 2014; Pettigrew 2008).

2.2 K^+ in soils

2.2.1 K^+ content in soils

K^+ concentration in soil solution may range between 0.025 and 5 mM (1 to 200 ppm) (Marschner 1995, Maathuis 2009). K^+ is an essential macronutrient for plant growth and development, constituting around 2.1–2.3% of the earth's crust (Schachtman & Shin 2007; Schroeder 1978). The soil mineral of this nutrient varies broadly (0.04–3%) (Sparks 1987; Sparks & Huang 1985), but even when the soil K^+ is high, most agricultural areas around the world are reportedly deficient in available K^+, including 75% of the rice (*Oryza sativa* L.) paddy soils of China and more than 60% of the wheat (*Triticum aestivum* L.) belt in southern Australia (Mengel & Kirkby 2001; Römheld & Kirkby 2010). The K^+ content in the soil depends on the nature of the soil; it is often low in sandy, waterlogged, saline, or acidic soils (Goulding & Loveland 1986; Zörb et al. 2014).

K^+ fertilisation has an effect on the physical properties of soil. It improves soil structure and the ability of the soil to capture water. It is reported that the application of mineral K^+ fertiliser improves the water holding capacity of the soil, and the structural stability of sandy soil in particular (Holthusen et al. 2010).

2.2.2 Soil K^+ and its availability to plants

Potassium availability differs significantly with the type of soil (Barré et al. 2008). There are four different pools of soil K^+: (1) soil solution K^+, (2) exchangeable K^+, (3) non-exchangeable

K^+ (located in the interlayers of clay minerals), and (4) lattice or structural K^+ (Barré et al. 2008; Moody & Bell 2006; Syers 1998). K^+ exchange amongst the pools depends upon macronutrient concentrations, pH in the soil and soil chemistry, and interacting factors that affect soil pH. K^+ deficiency is always associated with soil acidity where most of the cations including K^+ are leached during rainfall or in areas where crop production occurred for many years (Agenehu 2009; Kibebew & Usmael 2016; Yanai et al. 1996). Plant nutrient availability is greatly influenced by soil pH. However, the exchange capacities of cation and anion are directly affected by soil pH and soil with higher cations exchange capacities (CEC) than the anion exchange capacities (AEC), is able to bind more cation such as K^+ to the exchange site (Ann et al. 2009). A plants uptake of K^+ occurs entirely from the soil solution K^+ pool. This pool however is held in a dynamic equilibrium with both the exchangeable and the non-exchangeable pools (Moody & Bell 2006; Syers 1998). The exchangeable K^+ is rapidly released from exchange sites on the surfaces of clay minerals to replenish any shortfalls in K^+ in the soil solution (Steingrobe & Claassen 2000). Non-exchangeable K^+ however, can only be released into the soil solution once the soil solution K^+ concentration drops under 3.5µM (Springob & Richter 1998b). The weathering of clay minerals causes a slow release of structural K^+ into the soil solution with no effect during a single crop cycle (Pal et al. 2001b). Also, the presence of high levels of monovalent cations such as Na^+ and NH^{+4} may reduce plant K^+ levels because they interfere with K^+ uptake (Qi & Spalding 2004; Rus et al. 2004; Spalding et al. 1999). The soil minerals can also fix the K^+ in the soil by desorption of K^+ onto sites in the interlayers of weather sheet silicates. This is a fast process but K^+ may be released slowly depending on moisture content, competing ions, type of clay minerals and soil pH (Sparks 1987).

2.2.3 K^+ use in agriculture

Global K^+ demands in most agriculture regions has increased following the increase of population and climate change. Therefore, the major challenges for agriculture systems is to increase crop yield in a more efficient way (Reynolds et al. 2011). The use of fertiliser to provide adequate K^+ to production crops has increased by 25% since 1980. In just four years between 2011 and 2015, the demand for K^+ fertiliser increased from 23.8 Mt to 27.1 Mt (FAO 2011). This demand is projected to increase further to 37 million tonnes in 2020 (FAO 2015/2020). Cereal crops (wheat, maize (*Zea mays* L.) and rice) account for up to 37% of the

total K^+ fertiliser use; fruits and vegetables, 22%; oilseed 16%; and cotton 11%; with the remaining14% for various other crops (FAO 2011).

Potash is the major form of K^+ fertiliser used because it is the cheapest form. K^+ sulphate and K^+ nitrate is used for crops sensitive to chloride, including potatoes, and fruit crops such as bananas, citrus and grapes. Organic sources of K^+ fertiliser, such as animal manures, biogas residues and food waste are also used in agricultural systems (FAO 2011).

Generally, K^+ fertiliser is applied before planting most annual crops, and depending on soil type, one application is sufficient where K^+ is absorbed into clay minerals and is not leached. For light-textured soils to retain the K^+ from leaching and be more efficient, it is suggested that the application be divided into two or three applications (Annadurai et al. 2000). The safe range of K^+ application depends on the crop and soil type. Smaller seeds have less tolerance, with higher clay content and higher organic content in soils slightly reducing, the salt effect (Alberta 2013).

Table 2.1 World potash supply and demand balance, 2007/2008-2011/2012 (FAO 2008)

	2007/08	2008/09	2009/10	2010/11	2011/12
		K_2O (thousand tonnes)			
Total supply	38 325	37 512	39 526	41 474	43 213
Total demand	32 571	33 519	34 432	35 505	36 453
Surplus (deficit)	5 754	3 993	5 094	5 970	6 760

Fertilizer demand has generally been influenced by changing and often correlated factors, such as population and economic growth, agricultural production, prices and government policies (FAO 2011). Demand forecasts are based on agronomic considerations (e.g. cropped area and application rate of fertilizer), market feedback, estimates by industry associations, growth models, econometric models and expert advice (FAO 2015). The world potash supply fertiliser for the period 2015/2016 decreased by -1.8% but is expected to increase by 15.8 million tonnes (Mt) at an annual growth rate of 1.7 Mt (Table 2.2).

Potash fertiliser demand forecasts predict that East Asia will continue to account for by far the largest share of the world's potash fertiliser use in 2020, using almost twice as much as the next largest user, Latin America (FAO 2015). South Asia, North America and Europe are also predicted to be large users, though none of them are expected to use much more than half of what Latin America will (ibid). Africa, West Asia and Oceania (including Australia) are

expected to be comparatively small uses of potash fertiliser, even though the largest percentage increase of potash fertiliser use is expected to be in Africa (ibid).

Table 2.2 World global potash (K_2O), capacity, supply, demand and balance (non-fertiliser and fertiliser) and optimal fertiliser nutrients use and potential balance, 2015-2020 (thousand tonnes).

Year	2015	2016	2017	2018	2019	2020
World potash (K_2O)						
K_2O capacity	52 942	55 974	58 111	61 576	62 136	64 486
K_2O supply	43 571	42 772	44 868	47 249	48 898	49 545
K_2O non-fertiliser demand	5 626	5 524	5 586	5 654	5 720	5 886
K_2O demand fertiliser	32 838	33 149	34 048	34 894	35 978	37 042
K_2O potential balance *	5 107	4 100	5 233	6 701	7 200	6 617

FAO 2008

* Potential = supply – (non-fertilizer demand + fertilizer demand)

Table 2.3 World and regional potash fertilizer demand forecasts (thousand tonnes K_2O) and compound annual growth rate (CAGR) 2015 to 2020.

Region	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**CAGR (%)**
WORLD	32 838	33 149	34 048	34 894	35 978	37 042	2.44
AFRICA	647	662	708	765	838	897	6.76
North Africa	151	157	166	175	187	198	5.56
Sub-Saharan Africa	495	505	542	590	650	698	7.11
AMERICAS	11 589	11 833	11 977	12 129	12 487 1	12 830	2.05
North America	4 856	4 916	4 929	4 951	4 978	4 989	0.54
Latin America & Caribbean	6 733	6 917	7 048	7 178	7 510	7 841	0.00
ASIA	16 024	16 083	16 594	17 077	17 597	18 181	2.56
West Asia	260	276	291	308	326	347	5.91
South Asia	2 958	2 991	3 226	3 407	3 612	3 812	5.20
East Asia	12 805	12 817	13 076	13 362	13 659	14 023	1.83
EUROPE	4 187	4 193	4 390	4 539	4 669	4 741	2.52
Central Europe	650	650	700	750	780	800	4.24
West Europe	2 150	2 100	2 200	2 250	2 300	2 300	1.36
East Europe & Central Asia	1 387	1 443	1 490	1 539	1 589 1	1 641	3.42
OCEANIA	392	378	379	385	388	393	0.05

FAO 2008

The main potash producers are predicted to be Europe and North America, with West Asia also having surplus for export (FAO 2008).

Between the period of 1961 and 1998, the total output of K^+ in African countries almost doubled (30 kg K^+ ha^{-1} $year^{-1}$ through offtake) due to the level of imbalance between the supply and offtake (Sheldrick and Lingard 2004). K^+ fertiliser input has remained very low, at 2.1 kg K^+ ha^{-1} $year^{-1}$ or less, whilst other inputs (from crop residues and manures) have increased (Sheldrick & Lingard 2004; Manning 2010; FAO 2008; Moores 2009b). In Africa the demand for K^+ fertiliser will increase and will only be met by doubling the world production of potash fertilisers (Manning 2010). Approximately two-thirds of this growth was observed for Asia, and a quarter in America during the overlook period 2007//2008-2011/2012, while, on the last two years (2015/2016) Asia has half of the world potash demand 16 024/16 083 million tonnes respectively and expects to climb to 18 181 million tonnes by 2020, the annual growth rate (Table 2.3). America remained almost a quarter of the potash fertiliser in 2015, 11 589 million tonnes and will expect to use 12 830 million tonnes by 2020 refer to (Table 2.3).

Reducing the costs of K^+ fertilisers may be achieved by finding crop varieties more efficient in their use of K^+ (Rengel & Damon, 2008). Reaching this goal is likely only if we can identify and characterise the systems involved in K^+ acquisition in plants (Schroeder et al., 2013).

2.3 K^+ use efficiency (KUE)

2.3.1 Definitions

Some plant species and genotypes within species can grow and yield well on soils with low nutrient availability (Brennan & Bolland 2007; Damon & Rengel 2007a; Damon & Rengel 2007b; El-Dessougi et al. 2002; Gunes et al. 2006; Marschner et al. 2007; Rose et al. 2007; Yang et al. 2004). Potassium-efficient genotypes have the ability to produce high yields with low nutrient inputs and therefore have the capacity to increase the yield and sustainability of grain production systems on soils with mutable or low nutrient availability. K^+ efficiency is the percentage of yield potential that can be reached under K^+ deficiency (Damon, Osborne & Rengel 2007). High KUE species and genotypes tolerating nutrient deficiency (Rengel 2005; Rengel & Marschner 2005) have adapted physiological mechanisms which enable access to adequate levels of specific nutrients (uptake efficiency) and use that nutrient effectively (utilisation efficiency) (Sattelmacher et al. 1994). Therefore, the capability of genotypes to take up K^+ and yield well under conditions of low soil K^+ availability is described as a KUE (Damon & Rengel 2007).

2.3.2 Genetic variability in KUE among species and genotypes

Experiments have shown plants differ in their K^+ efficiency; some plant species gain higher yields despite a low soil resource, whereas other species fail (Meyer & Jungk 1993; Sadan & Claassen 1999; Steingrobe & Claassen 2000; Trehan & Claassen 1998; Zhang et al. 1999). There is a strong relationship between uptake and utilization efficiency to produce K^+-efficiency (Rengel & Damon 2008). This efficiency can be due to different mechanisms, such as root morphology, formation of root hairs, root exudates, ability to release K^+ from non-exchangeable pools, kinetics of K^+ uptake, K^+ translocation, K^+ substitution and harvest (White 2013). (Pettigrew 2008) reported that the first five mechanisms listed above are related to K^+ uptake efficiency, while the latter three determine K^+ utilisation efficiency.

2.3.2.1 Root morphology

It is important to have a large surface area between the root and the soil for diffusion-supplied K^+. Hence, K^+-efficient genotypes have a greater proportion of thin root in their root system

compared with K^+-inefficient genotypes. Fast root turnover may also be crucial in K^+ uptake for many genotypes under low K^+ availability (Wong et al. 2000).

K^+ leaching, particularly in sandy soils, shows a significant loss of K^+ and supports genotypes that grow long root systems to uptake K^+ from deep in the soil; it therefore may be beneficial to recycle K^+ through the soil–stubble continuum (Wong et al. 2000). However, contrasting genotypes shows K^+-efficiency may not be linked to increased root growth. K^+-efficient genotypes, such as potato, had half the root length of the K^+-inefficient plants, even though they have same shoot growth. The K^+-efficient plants had higher K^+ influx than K^+-inefficient plants (Trehan & Sharma 2002). A mechanistic model calculates the K^+ uptake and supply level of the soil by the diffusive and convective transport of nutrients towards the root under absorption and desorption processes. Thinner root hairs create a sharper diffusion gradient through increased surface area and K^+-depletion zone, which can contribute to the K^+ uptake capacity (Jungk 2001; Steingrobe & Claassen 2000).

2.3.2.2 Formation of root hairs

Root hair length was positively correlated with capacity for K^+ uptake from deficient soils for pea, red clover, barley, rye, perennial ryegrass and oilseed rape (Hogh-Jensen & Pedersen 2003). Root hair is characterized as thinner than the root which make it easy to create a steeper diffusion gradient. The root hair characteristics can differ between genotypes in the same species (Jungk 2001). It was also shown the high K^+ uptake efficiency in wheat is mainly due to its large root system.

2.3.2.3 Root exudates and K^+ mobilization from non-exchangeable pool

The difference in root exudation between different genotypes leads to a disparity in K^+ efficiency (Trehan 2005a). This is because enhanced organisation of mineral K^+ can be explained by the release of organic acids (Wong et al. 2000). The exudates of soil microorganisms can also facilitate K^+ release from the clay minerals, by excreting organic acids (Sheng et al. 2002) which dissolve K^+-containing primary minerals such as rock K^+ or chelate them.

K^+ can be brought into solution by the primary minerals (Basak & Biswas 2009; Bennett et al. 1998). The major compounds released include organic acids such as citric and oxalic acids in maize (Kraffczyk et al. 1984), tartaric acid in pak choi and radish (Chen et al. 2000), and malic

acid in oilseed rape (Zhang et al., 1997). Similarly, amino acids, which are found in root exudates of wheat and sugar beet, enhance K^+ release from clay minerals (Rengel & Damon 2008).

It has been reported that the variation in K^+ efficiency among 10 potato cultivars was due to the differential uptake from non-exchangeable K^+ from the soil (Trehan 2005a). Efficient genotypes obtained 46% more K^+ from the non-exchangeable pool, while the inefficient genotypes obtained only 17–25% (Trehan 2005). This result indicates the ability to utilize non-exchangeable K^+ is an important factor (Bairwa 2010; Trehan & Sharma 2002). It was suggested that detected genotype exudation of K^+-mobilising compounds from the root accounts for the differential uptake of non-exchangeable K^+ (Trehan 2005a). For example, assessments of experimental data indicate wheat was K^+-efficient because of the high root–to-shoot ratio, while sugar beet increased K^+ availability in the rhizosphere (Steingrobe & Claassen 2000). It has been shown that non-exchangeable K^+ can also be used by plants when the available fraction is too low for a sufficient supply (Claassen & Steingrobe 1999). Some types of bacteria can also play an active role in increasing K^+ uptake by extracting K^+ from the non-exchangeable pool (Supanjani et al. 2006). For example, *Bacillus edaphicus* releases K^+ from illite and can be found in the rhizosphere of cotton plant, rape (Sheng 2005) and in wheat (Sheng & He 2006).

2.3.2.4 Kinetics of uptake

The root's ability to take up K^+ at high rates, despite low soil K^+ concentrations, can greatly influence K^+ uptake efficiency (Springob & Richter 1998b). Under low K^+ conditions, wheat genotypes differed in their K^+ uptake, but in soil with high K^+ fertilization the K^+ uptake per unit of root length is higher in the K^+-efficient genotypes (Glass & Perley 1980).

In some species, the rate of K^+ uptake is an important mechanism of K^+ uptake efficiency. For example, wheat and sugar beet required a nine-times lower external K^+ concentration than potato, to achieve 90% of maximum yield in a flowing nutrient solution culture. The K^+-efficient potato genotypes grew two-fold more than K^+-inefficient genotypes at K^+ deficiency (Trehan & Sharma 2002).

2.3.2.5 Translocation

The differences between genotype efficiency of K^+ utilization is also related to differences in the capacity to translocate K^+ at a cellular and plant level, due to the expense of vacuole K^+ activity under K^+ deficiency, so that the cytosolic activity can be maintained (Leigh 2001; Memon et al. 1985). However, in root and leaf cells, the activity of K^+ in vacuoles is regulated differently than in the cytosol (Cuin et al. 2003). In K^+-efficient barley genotypes, the movement of K^+ from the vacuole into the cytosol was stronger than in K^+-inefficient genotypes (Memon et al. 1985).

Translocation of K^+ between the organs within the plant is one of the most important mechanisms in K^+ efficient utilization (Dunlop & Tomkins 1976). The capacity of a genotype to produce high yield is affected by its capacity to translocate K^+ from non-photosynthetic organs, such as the stem, to upper leaves and harvested organs. In rice, two K^+-efficient genotypes had a two-fold higher K^+ concentration in lower leaves, but in the upper leaves the K^+ concentration was only 30% higher compared to the K^+-inefficient genotypes. To maintain a high photosynthetic rate during grain filling, K^+-efficient genotypes need to maintain the high K^+ concentration in the lower leaves (Yang et al. 2004).

2.3.2.6 Substitution

Cell extension requires high mobility of the osmolytes, and in turn requires vacuolar K^+ to create osmotic potential so only Na^+ can replace K^+ in this role (Amtmann et al. 2005). Also in enzymatic reactions, Na^+ can replace K^+, which leads to organic acid production (Ehrendorfer 1973). Na^+ can be a partial substitute for K^+ in some (especially halophyte) but not all species (Marschner 1995).

Under K^+ deficiency, some tomato genotypes differ in regard to their growth response to substitution capacity for Na^+ (Figdore et al. 1989). This indicates the capacity of genotypes to use Na^+ as a substitute for K^+ is greater in salt-tolerant genotypes (i.e. *Lycopersicon pennellii*) than in the cultivated tomato (Taha et al. 2000).

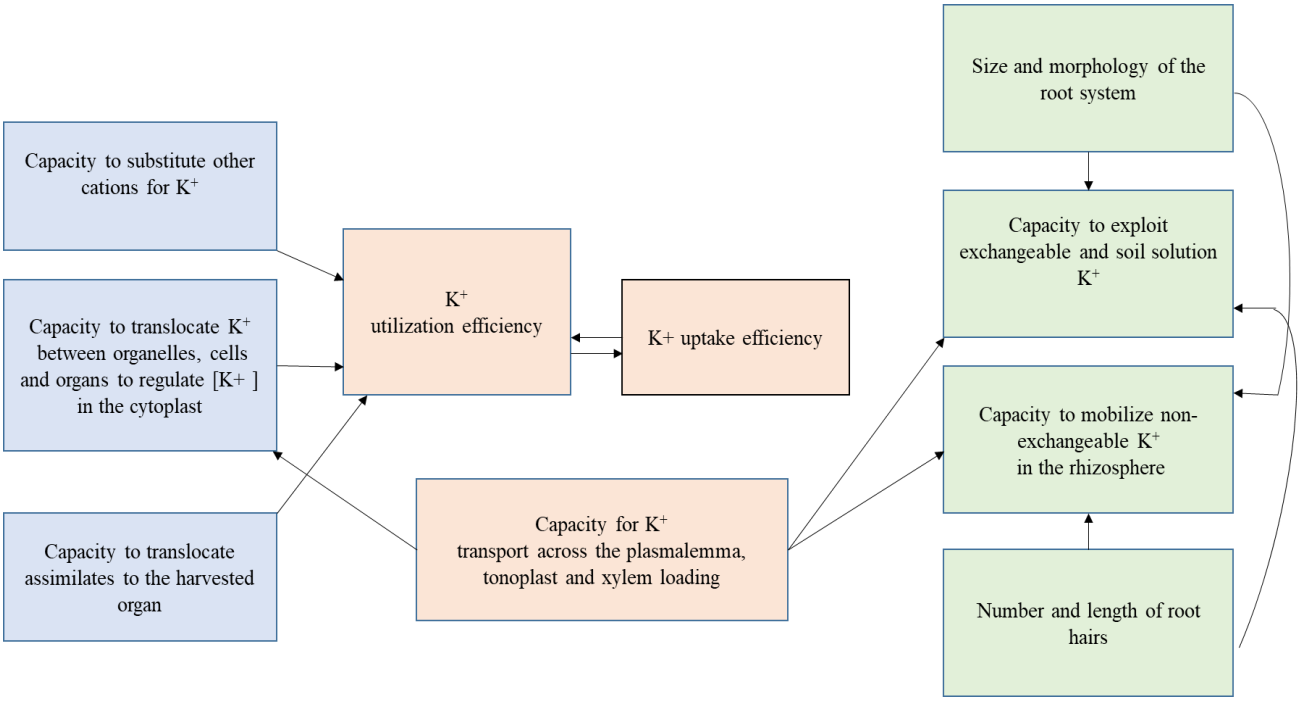

Figure 2.1 Probable mechanisms of K^+ uptake and utilization efficiency and their exchanges that influence the expression of the K-efficient phenotype (Rengel 2008).

Table 2.4 Methods of evaluating plant KUE (Rengel & Damon 2008)

Crop species	Genotypes tested	Environment	K rate applied	Growth stage (Zadok)	Suggested Parameter for differences in K^+ efficiency	Reference
Sweet potato (*Ipomoea batatas L.)*	108	Field	300 kg ha^{-1} K_2SO_4	Maturity (87 Z)	Root dry matter yield and biomass yield, and in K^+ concentrations, accumulations and use efficiencies	Wang et al. 2015
	7	Field	0, 150, 300, and 450 kg ha^{-1} K_2SO_4	Maturity (87 Z)	Tuberous root dry matter, tuber weight. K^+ accumulation levels in the seven elite sweet potato genotypes peaked under a deficient K^+ supply at 100 days after plantation and decreased	
Wheat *(Triticum aestivum L.)*	154	Glasshouse	0 or 88 mg kg^{-1}	20-29 Z	The ratios at maturity for grain yield, shoot weight, harvest index, grain yield (mg/pot) at deficient K^+ supply and adequate K^+ supply. The K^+ efficiency ratio for shoot weight and shoot K^+ concentration (mg/g) at deficient K^+ supply.	Damon and Rengel (2007)
	12	Glasshouse	15 or 100 mg kg^{-1}	Zadok 3.1 (stem elongation) and maturity (87 Z)		
	88	Field	0 or 100 kg ha^{-1}	Zadok 4 and maturity (87 Z)		
	58	Field	Nil K^+ only	Maturity (87 Z)		Zhang et al. (1999)

	4	Field	0 or 111 kg ha $^{-1}$	Maturity (87 Z)	Shoot and grain yield per unit of K^+ taken up, harvest index	
	3	Glasshouse	0–10 mM K	Tillering (20-29 Z)		
	21	Field	83 kg ha $^{-1}$	Maturity (87 Z)	Shoot and grain yield per unit of K	El-Bassam (1998)
	16	Growth room and natural condition	10 µM K	20-29 Z and maturity (87 Z)	Harvest index, shoot/grain yield per unit of K^+ taken up	Woodend et al. (1987)
Rice *(Oryza sativa L.)*	134	Glasshouse	5 or 40 mg l $^{-1}$	Maturity (87 Z)	Shoot and grain yield per unit of K^+ taken up, harvest index	Yang et al. (2003)
	9	Field	0 or 78 kg ha $^{-1}$	Tillering (20-29 Z) and maturity (87 Z)	Shoot and grain yield per unit of K taken up, harvest index	
	9	Field	157 kg ha $^{-1}$	Maturity (87 Z)	Harvest index, shoot/grain yield per unit of K^+ taken up, translocation of K^+ to upper leaves	Yang et al. (2004)
Barley (*Hordeum vulgar L.)*	10	Growth room	0.1–500 µM	2 weeks	K influx rate	Glass and Perley (1980)
	11	Growth room	3 mM	Young seedling	Shoot yield per unit of K^+ taken up	Jensen and Pettersson (1980)
Canola (*Brassica napus L.)*	84	Glasshouse	0 or 88 mg kg $^{-1}$	37 days old	Correlation between content and concentration of K^+ in shoot dry weight with deficient K^+ and adequate K(88mg/kg)	Damon et al. (2007)

Potato *(Solanum tuberosum)*	10	Natural conditions	0 or 195 mg kg^{-1}	29 and 83 days	Uptake of non-exchangeable K, root length/shoot ratio	Trehan et al. (2005)
	3	Natural conditions	0 or 100 mg kg^{-1}	19 and 45 days	Uptake of non-exchangeable K, K^+ influx rate	Trehan and Sharma (2002)
Common bean *(Phaseolus vulgaris L.)*	10	Glasshouse	0 or 200 mg kg^{-1}	Maturity (87 Z)	K partitioning, harvest index	Baligar et al., (2001)
Tomato *(Lycopersicon esculentum Mill.)*	4	Growth room	nil K^+ only	3 weeks	Root length, high K^+ influx	Chen and Gabelman (2000)
	5	Growth room	5 mg pot^{-1}	30-40 days old	Na/K substitution capacity	Figdore et al. (1989)
Sweet potato *(Ipomoea batatas L.)*	8	Field	0 or 112 kg ha^{-1}	150 days	Root and total biomass yield per unit of K^+ taken up	George et al. (2002)
Lucerne *(Medicago sativa L.)*	9	Glasshouse	0, 100 or 200 mg kg^{-1}	Early bloom	Shoot yield per unit of K^+ taken up	James et al. (1995)

2.4 Factors determining KUE

2.4.1 Root membrane transporters mediating K^+ uptake from the soil

A plant's K^+ requirement needs to be met through the uptake of K^+ from the soil solution (Gierth & Mäser 2007). Plant roots take up K^+ through the epidermal and cortical cells and then distribute it to other organs, such as shoots and leaves (Nieves-Cordones et al. 2014). K^+ is transported across the membrane via either passive (channels) or active (co-transporters) transport systems, depending on the electrochemical gradient (Gierth & Mäser 2007).

There are two systems of K^+ transporting, the high-affinity system (HATS) that operates at low external concentrations, and the low-affinity system (LATS) at higher concentrations (Epstein et al. 1963). The HAK1 transporter from KT/HAK/KUP family (Figure 2.4) is a major candidate for HATS in some species, such as barley (Santa-María et al. 1997), rice (Ba˜nuelos et al. 2002) and pepper (Martínez-Cordero et al. 2004). In other species such as *Arabidopsis* (Rubio et al. 2000) or tomato (Nieves-Cordones et al. 2007) this role is attributed to HAK5. These transporters are primarily expressed in the roots and play an important role in K^+ uptake from the external environment (Gierth et al. 2005; Hirsch et al. 1998; Lagarde et al. 1996). AKT1 from the Shaker family is known as an inwardly rectifying K^+ channel for LATS (Lagarde et al. 1996). These two types of transport proteins mediate almost all K^+ absorption in *Arabidopsis* roots (Gierth et al. 2005). In *Arabidopsis* HAK5 and AKT1 mediate K^+ uptake at external concentrations ranging from 10 to 200µM, AtHAK5 can even uptake below 10µM; when the external K^+ concentration is sufficiently high, AKT1 contributes to K^+ uptake probably together with non-selective cation channels (NSCCS) (Alemán et al. 2011; Caballero et al. 2012).

The optimal K^+ concentration for sufficient metabolic functions in the cytosol of plant cells is around 100 mM (Britto & Kronzucker 2008; White & Karley 2010). The K^+ concentrations of the soil solution are highly mutable, usually in the range of 1 to 0.1 mM, and sometimes lower (Maathuis 2009). K^+ ions enter the root cell against its concentration gradient; the plasma membrane of plant cells is invigorated by the activity of a H^+-ATPase. The resulting H^+ gradient (Δ pH) (pH_{ext} = 5.5, pH_{cyt} = 7.3) creates a negative electrical gradient (ΔV_m) inside. K^+ as a cation can be passively driven into the cell down the electrochemical gradient and

pumps H^+ out of plant cells. This process can occur by a secondary transport system, such as a uniport (for example, the inward-rectifying K^+ channel AKT1). The membrane potential of a plant cell is also greatly affected by the depolarisation as a result of K^+-uptake through these channels, and depends on the external K^+ concentration (Wang et al. 2010). The values of the K^+ gradient and the (ΔV_m) across the plasma membrane limit the ability of the K^+ channel to concentrate K^+ in the cell. A summary of membrane transporters involved in the uptake of K^+ and transport in plants and the structures they are expressed in are presented in the table below (Table 2.5).

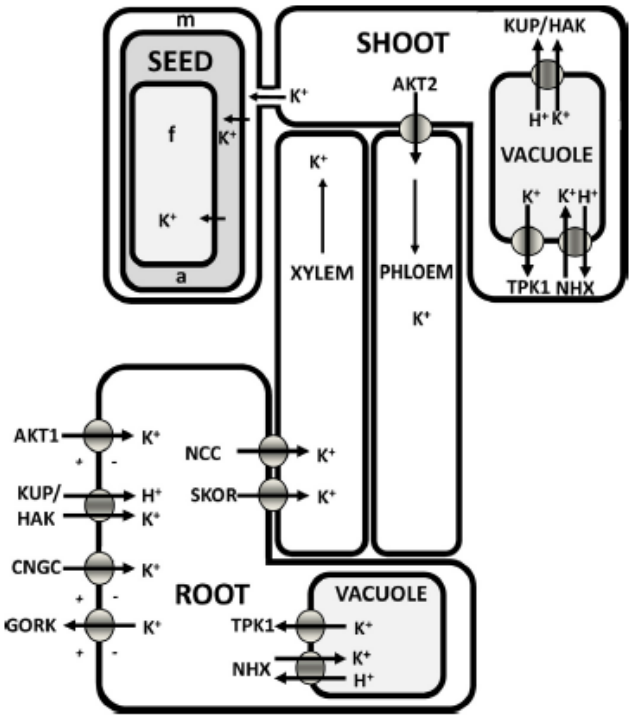

Figure 2.2 Overview of the K^+ transport processes and transporters involved in uptake, efflux and distribution within the plant. At the soil-root interface both active (KUP/HAK) and passive (AKT1, CNGC) transporter are responsible for K^+ uptake, while GORK mediate Based on the knowledge of electrophysiological analyses in plants by heterologous systems and reverse-genetics approaches, K^+ transport activity channels were found to be regulated by various proteins, such as kinases, phosphatases, 14-3-3 proteins, syntaxins, farnesyl transferase and G proteins (Assmann 2002; Blatt 2000; de Boer 2002; Schroeder et al. 2001). The other two effectors that can regulate K^+ transport systems due to their vital roles in regulating guard cell inward and outward K^+ rectifiers (Blatt 2000; Blatt & Grabov 1997) are voltage and pH (Kochian & Lucas 1988).

Table 2.5 K^+ channel and transporters in Arabidopsis (adapted from Gambale & Uozumi 2006; Gierth et al 2007; Grabov 2007; Lebaudy et al. 2007; Very & Sentenac 2003).

Family/name	Protein structure	Organ(s)/tissue(s)	Functions	Reference(s)
Shaker				
AKTI	6 TMSs, 1 P-loop, CNBD, Anky, KHA	Root (root hair, epidermis, cortex), leaf (primordial, mesophyll, hydathodes, guard cell)	Inward-rectifying K^+ channel, K^+ uptake into root cells	Gierth, M. et al. 2005; Hirsch, R. E. et al. 1998; Lagarde et al. 1996; Lee, SC et al. 2007; Li et al. 2006; Pyo et al. 2010; Sentenac et al. 1992; Spalding et al. 1999; Xu et al. 2006
SKOR		Root (stellar tissue), pollen	Outward-rectifying K^+ channel, K^+ release into xylem, K^+ translocation from roots to shoots	Gaymard et al. 1998
KATI		Leaf (guard cells)	Inward-rectifying K^+ channel, K^+ uptake into guard cells, stomatal regulation	Pilot et al. 2001; Szyroki et al. 2001
SPIK		Pollen (pollen, pollen tubes)	Inward-rectifying K^+ channel, K^+ uptake into pollen tubes, pollen tube development regulation	Mouline et al. 2002
AKT2		Root (phloem stem, leaf (phloem, mesophyll, epidermis, guard cell), Flower (sepal)	Weakly rectifying K^+ channel, K^+ circulation in phloem	Chérel et al. 2002; Gajdanowicz et al. 2011; Lacombe et al. 2000; Marten et al. 1999; Michard et al. 2005
KATI		Leaf (guard cells)	Inward-rectifying K^+ channel, K^+ uptake into guard cells, stomatal regulation	Anderson et al. 1992; Kwak et al. 2001; Schachtman et al. 1992; Sottocornola et al. 2006; Sutter et al. 2006; Szyroki et al. 2001

KAT2		Leaf (guard cells, phloem)	Inward-rectifying K^+ channel, K^+ uptake into guard cells, stomatal regulation	Pilot et al. 2001; Szyroki et al. 2001
GORK		Root (root hairs, epidermis), Leaf (guard cells)	Outward-rectifying K^+ channel, K^+ release from guard cells, stomatal regulation	Ache et al. 2000; Hosy et al. 2003
AtKCI		Root (root hair, epidermis, cortex), leaf (epidermis, hydathodes, trichome)	Silent K^+ channel, assembly with Shaker Inward K^+ channel and regulation of K^+ uptake into 102cells	Dreyer et al. 1997; Duby et al. 2008; Geiger et al. 2009; Jeanguenin et al. 2011; Reintanz et al. 2002; Wang et al. 2010
TPK				
TPK1	4TMSs, 2 P-loops. EF hand, 14-3-3 binding	Roots (root tip, elongation zone), leaf (mesophyll, guard cells), flower (sepals, anthers, pollen)	Vacuolar K^+ channel, K^+ release from vacuole, stomatal closure, intracellular K^+ homeostasis	Czempinski et al. 2002; Gobert et al. 2007; Latz et al. 2007; Voelker et al. 2006
TPK4		Root (root hairs, epidermis), leaf (guard cells	PM K^+ channel, control of pollen PM voltage	Becker et al. 2004; Voelker et al. 2006
KUP/HAK/KT				
KUP1	10-14 TMSs	Steam, leaf, flower	Dual-affinity K^+ transporter, K^+ uptake into cells	Fu & Luan 1998; Kim et al. 1998
KUP2		Root (root tip), stem (hypocotyls, inflorescence steam), leaf (cotyledons, young leaves)	Low-affinity K^+ transporter, K^+ dependent cell expansion	Elumalai et al. 2002; Fu & Luan 1998
KUP4		Root, steam leaf, flower	High-affinity K^+ transporter, K^+ translocation and root hair	Fu & Luan 1998; Rigas et al. 2001; Vicente-Agullo et al. 2004

HAK5		Root (epidermis, stele)	High-affinity K^+ transporter, K^+ uptake into root cells	Gierth, M. et al. 2005; Pyo et al. 2010; Qi et al. 2008
NHX				
NHX1/NHX2	10-12 TMSs	Steam (cortical tissue), leaf (vascular tissue, guard cells), (flower (petals, stamens, anthers), silique (developing embryo)	Vacuolar Na^+ (K)/H^+ antiporter, vacuolar PH and K^+ homeostasis, turgor regulation, plant growth, flower development, stomatal function	Apse et al. 2003; Barragán et al. 2012; Bassil et al. 2011; Venema et al. 2002
CHX				
CHX13	10-12 TMSs	Root, Flower, Pollen	High affinity K^+ transport, K^+ uptake into root cells	Zhao et al. 2008
CHX17		Root (epidermis, cortical cells)	Na^+ (K)/H^+ antiporter, K^+ uptake into root cells and K^+ homeostasis	Cellier et al. 2004
CHX20		Leaf (guard cells)	Na^+(K)/H^+ antiporter, K^+ homeostasis and pH regulation, guard cell osmoregulation	Padmanaban et al. 2007
CHX21		Pollen (pollen, Pollen tube)	Na^+(K)/H^+ antiporter, pollen tube targeting to ovules	Lu et al. 2011
CHX23		Root, stem, leaf (cotyledons), flower (sepals), pollen	Na^+(K)/H^+ antiporter, pH homeostasis and chloroplast development, pollen tube targeting to ovules	Lu et al. 2011; Song et al. 2004

Abbreviations: 14-3-3 binding, 14-3-3 protein binding domain; Anky, ankyrin domain; CNBD, putative cyclic nucleotide-binding domain; EF hand, EF hand domain; KHA, domain rich in hydrophobic and acidic residues; P-loop, pore-loop domain; PM, plasma membrane; TMS, transmembrane segment domain. The expression profiles of the K^+ channels and transporters in different organs, tissues, or cell types.

2.4.2 Regulation of K^+ loading into the xylem

Several factors regulate K^+ loading into the xylem. These factors could be voltage gating, external K^+, molecular structure of the channels, hormonal regulation, Na^+ removal from the xylem, cytosolic Ca^{2+} and internal and external pH. The SKOR and GORK channels are outward rectifying channels regulated by voltage. SKOR activity is also affected by external K^+ (Gaymard et al. 1998) where a high outward current was observed under low external K^+ condition. This phenomenon is explained as allosteric regulation, which is found in other outward rectifying channels from plants and animals (Bruggemann et al., 1993; Pardo et al., 1992). Johansenn et al (2006) identified that the S6 and pore domain are responsible for sensing external K^+ in SKOR. K^+ secretion into the xylem is shown to be under strict hormonal regulation. ABA (abscisic acid) treatment showed a transient reduction of the mRNA level of AtKC1, a subunit contributing to K^+ in channels and a strong (90%) reduction in mRNA level of SKOR (Gaymard et al. 1998; Pilot et al. 2003). The effect of benzyladenine (BA) treatment was even stronger with no mRNA detected for 12 h after 50µM BA treatment (Pilot et al., 2003). Na^+ removal from the xylem sap stimulates K^+ loading to the xylem resulting in increased K^+ accumulation in the shoot. SKOR channel activity is also dependent on cytosolic Ca^{2+} and internal and external pH (Lacombe et al, 2000a). A relatively small decrease in cytosolic pH from 7.4 to 7.2 has resulted in a large (80%) reduction in voltage independent macroscopic SKOR current in a heterologous expression system in oocytes (Lacombe et al., 2000a). This is different from the inward channel KAT1 whose activity is enhanced by lowering the pH from both sides of the membrane (Hoth & Hedrich 1999).

2.4.3 Importance of K^+ cycling for KUE

K^+ is also essential for phloem sugars loading and translocation (Patrick et al. 2001). In plants all the soluble movement of photosynthates occur through the phloem from leaves to roots and other sink organs; but the redistribution of K^+ toward growing tissues, such as developing leaves and fruits, is normally from older to younger plant tissue through the phloem (Mengel et al. 1987).

K^+ circulating in the phloem acts as a decentralised energy store which is utilized when the local energy is limited. Post-translational amendment of the phloem is expressed by K^+ channel AKT2 (*Arabidopsis*), which supports the plasma membrane H^+-ATPase in activating the transmembrane

phloem as a reloading process; after that, the K^+ re-translocate from the shoot back into the root via the phloem. The consequent reloading back into the xylem occurs in the case of K^+ delivery more than shoot requirements or under conditions of K^+ deficiency (Jeschke & Hartung 2000). K^+ accumulation is essential for facilitating high transport rates by establishing and maintaining a high osmotic potential in the sieve tubes, as K^+ plays an important function in the solute transport of phloem sieve tubes (Marschner 2012).

K^+ is highly mobile within plants, exhibiting long-distance cycling between roots and shoots in the xylem and phloem. This is most evident in the cotransport of K^+ with nitrate (NO_3) to shoots and its subsequent re-translocation to roots with malate when plants are supplied with NO_3, and is also seen in the cotransport of K^+ with amino acids in the xylem (Du et al. 2015).

2.4.4 K^+ delivery and metabolisation in the shoot

K^+ is taken up from the root and passed to xylem by unloading K^+ into xylem and loading to phloem for distribution into photosynthetic and non-photosynthetic tissues for metabolisation. Xylem is the main route where nutrients are transported from soil to the above ground shoot for metabolisation. K^+ is important for turgor and hence, expansion growth. Therefore, large amounts of K^+ should be unloaded from xylem to the shoot (Ahmad & Maathuis 2014). K^+ requirement varies, for example between mature and transpiring leaves and growing young leaves. This process is controlled by various hormonal signals such as auxin, ABA, cytokinins and many of them can be found in the xylem itself (Hu et al. 2016; Wegner & De-Boer 1997). The xylem parenchyma has interruptions in the cell walls (pits) that directly get in contact with the content of the xylem. The xylem K^+ is unloaded to the apoplast and passively taken up by xylem parenchyma and symplastically transferred to other nearby or distant tissues (Botha et al. 2008). The molecular mechanism behind these transport processes is not well studied, but it is believed K^+ channels and carriers are involved.

Phloem K^+ concentrations are usually higher than the xylem K^+ concentration and can reach 50-150 mM (Kallarackal et al. 2012). The relatively higher concentration of K^+ in the phloem is not clear and could be due to many factors. The loading of sugars and amino acids are stimulated by high apoplastic K^+ (Chen et al. 2015; Dreyer et al. 2017; Peel & Rogers 1982). Phloem contains

large amounts of negatively charged organic acids and amino acids (Hayashi & Chino 1990). Similarly, phloem K^+ serves as a charge balance as it does in the xylem.

K^+ concentration varies within shoot tissues in photosynthesising mesophyll and non-photosynthesising epidermal tissue. The variation is mainly in the vacuolar content. Under controlled conditions, the K^+ concentration showed 200mM in both mesophyll and epidermal vacuoles. However, changing the growth conditions affected K^+ concentration in both tissues, but the epidermal vacuole concentration was more affected (Fricke et al. 1996; Wu et al. 2015). A study on barley showed a K^+ concentration of ~260 mM in epidermal cells compared to ~120 mM in mesophyll cells. Another finding obtained the opposite, K^+ concentration of ~300 mM mesophyll cells and ~ 170 mM in epidermal cells (Dietz et al. 1992). This indicates that the partitioning depends on the stage of growth and development (refer to Figure 5 below).

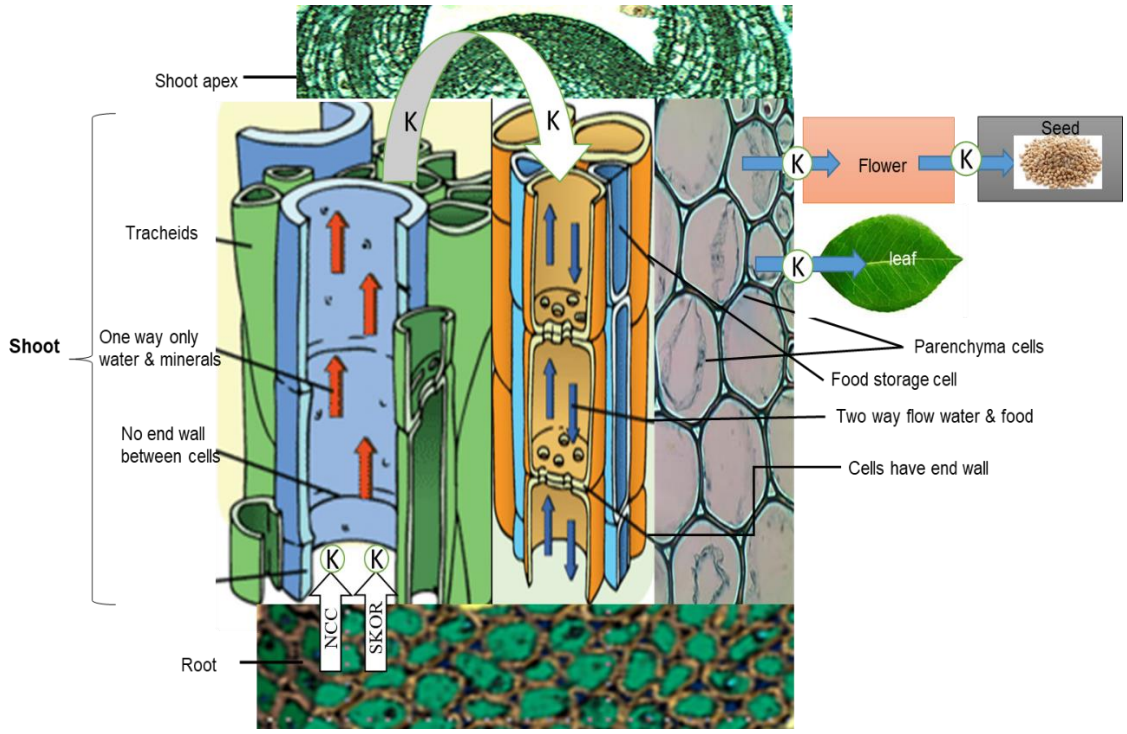

Figure 2.3 Overview the processes of K^+ uptake by the roots through K^+ selective (SKOR) and non-selective (NCC) cations channels, phloem loading of K^+ for recycling and K^+ flux to the leaf, flower and seed through parenchyma cells.

2.4.5 Impact of source-sink interaction for KUE

The K^+ concertation in the soil solution is several orders of magnitude lower than in the plant. Therefore, plants have to invest a considerable amount of energy to take up K^+ (Ma et al. 2012). Roots cell take up K^+ across plasma membranes against a concentration gradient using membrane transporter and channels proteins (Chérel et al. 2014; Rogiers et al. 2017).

K^+ is taken up by the cells of developing shoots through long-distance transport along the xylem (Marschner et al. 1997). From the mature (source) leaves, photosynthates and K^+ are allocated via the phloem to sink tissues (Jeschke & Pate 1991; Lalonde et al. 2003) and used for growing leaves, developing flowers, ripening fruits and seeds. (Ache et al. 2001; Marschner, H et al. 1996; Rogiers et al. 2017).

The movement of sucrose from the sinks to the sources requires higher phloem K^+ levels. This is crucial for proper root development and growth, but since seed filling primarily takes place via the phloem, K^+ homeostasis affects the protein content of the grain (Meharg 2012).

K^+ channels control the movement of plant K^+ from source to sink through the phloem. This process is achieved by the adaptation of K^+ channels according to the sink/source relation, fructose, and pH concentration (Ache et al. 2001).

2.5 Impact of environmental factors on KUE

2.5.1 Salinity

Salinity is an environmental problem affecting crop production around the world; up to 7% of the total land surface is saline (Elphick et al. 2001; Flowers & Yeo 1995; Haw et al. 2000). A plant's growth effected by salinity is due to the early injuries in the first stage and ionic injuries in the later stage (Sanadhya et al. 2015). The effect of salinity on photosynthesis is complicated; low salinity can in fact enhance photosynthesis (Greenway & Munns 1980), due to the effect of mild salt causing an increased K^+ uptake (Chen et al. 2005), also later research shows exchanging K^+ supply with suitable amounts of Na^+ could be beneficial for cotton yield (Zhang et al. 2006). High salt levels impede plant growth by inducing osmotic and ionic stress (Shabala & Cuin 2007) and decreasing the photosynthetic performance (usually related with leaf chlorosis) (Crillo et al. 2011; Bethke & Drew 1992; Seemann & Critchley 1985). A high concentration of both K^+ and Na^+ cations in the soil leads these two ions competing and results in reducing their uptake of K^+ by plants (Zhu 2003), and a high Na^+ level across the plasma membrane causes significant membrane depolarisation (Shabala et al. 2005) resulting in K^+ loss. Using halophytic species is one of the promising ways to overcome this problem, as they can grow ideally at 100-200 mM NaCl, and they have high resistance to environmental stress and are high in nutritional quality (Becker et al. 2017; Sun & Jacobsen 2013; Sun et al. 2017; Sun et al. 2014). With halophytic species being better able to preserve the K^+ content under salinity conditions, they have the ability to use Na^+, Cl^- as inorganic ions to maintain cell turgor and sequestration of these cytotoxic ions into the vacuole under salinity (Flowers. & Colmer 2008; Shabala & Mackay 2011). This process requires adequate amounts of K^+ to increase the osmatic potential, as K^+, is the major osmotic, and can contribute 35–50% of cell osmotic potential (Rivelli et al. 2002). It has also been reported that

adding K^+ fertiliser has a positive impact on plant performance under saline conditions (Umar et al. 2011).

Salt-tolerant plants have the capacity to maintain higher ratio of K^+/Na^+ in the cytosol (Maathuis & Amtmann 1999; Shabala & Cuin 2008). Under saline conditions, plants also suffer from increased ROS production (He et al. 2015; Shabala & Pottosin 2014; Bose et al., 2014) the effect is worse in cases of severe K^+ deficiency. Higher K^+ nutritional status of plants reduces the ROS production by enhancing photosynthetic electron transport, and obstructing the membrane-bound NADPH (Cakmak 2005).

Salinity may have an indirect effect on plant photosynthesis by reducing stomatal conductance due to the osmotic component of salt stress (Munns 2002). As mentioned before, K^+ deficiency reduces photosynthesis through its function in stomata regulation with K^+ deficiency increasing stomatal resistance to CO_2; so that under salinity conditions more fertiliser will be needed. Most plant species including barley (Chen et al. 2005) shows a severe K^+ deficiency in the shoots under salt stress (Marschner 1995). This can be explained by the dramatic decrease in the transpiration rate induced by salinity as revealed by the measurement of stomatal conductance (Shabala et al. 2005). However, this reduction of shoot K^+ concentration under salinity conditions is less severe in the tolerant species, because they can maintain a much higher shoot K^+ content under saline conditions (Chen et al. 2005; Chen et al. 2007a).

Under salinity stress, the growth of many plants is limited, partially due to insufficient water availability, but also as a result of ion toxicity. The cytosol of these plant cells does not tolerate Na^+ concentrations greater than 20 mM (Alberts et al. 2007; Amtmann & Sanders 1999; Blumwald & Aharon 2000; Walker et al. 1996). While some plants appear to utilize Na^+ to certain concentrations, it is problematic, if the concentration of Na^+ becomes high (Kronzucker et al. 2013). Many halophytic plants take advantage of the similarity between Na^+ and K^+ for uptake and grow in high salt environments, by reducing the K^+ requirement of meeting basal metabolic requirements. Non-halophytic plants under limited K^+ supply, can utilize Na^+ together with Mg^{2+} and Ca^{2+} to replace K^+ in the vacuole as an alternative inorganic osmotic (Flowers & Läuchli 1983; Kronzucker & Britto 2011). In this case, the released K^+ is available for more K^+-specific processes.

2.5.2 **Waterlogging**

Controversial reports were presented in the literature for the changes in shoot and root K^+ content under flooding conditions. Shoot K^+ content dropped several fold in some plants, with no change or even increase in K^+ in roots due to the complexity of the root to shoots nutrients translocation under waterlogging stress (Ashraf & Rehman 1999; Close & Davidson 2003; Smethurst et al. 2005; Teakle et al. 210). The waterlogging impact on K^+ content in the shoot depends on the period of the treatment and species. Xylem sap osmolality and K^+ concentration increase in flooded plants (Ou et al. 2011; Pal et al. 2004; Polacik & Maricle 2013). This is due to a decline in stomatal conductivity, which affects the nutrient delivery to the shoot in long periods of flooding (Jackson et al. 1996; Ou et al. 2011; Pang et al. 2004).

The availability of oxygen plays a substantial role in K^+ uptake in flooded roots, depending on whether they are deprived of oxygen, or have a shortage of oxygen, species with oxygen deprivation reduce K^+ uptake (Elzenga & Veen 2010), so under hypoxia the K^+ uptake could be highly reduced or stop entirely (Kuiper et al. 1994). This variation is due to the viability of alteration in the metabolic K^+ pool to maintain the membrane potential and reactivate the K^+ low affinity transport (Morard et al. 2004). Plant species act differently under these conditions with sensitive species experiencing a disruption of ion homeostasis and cell death due to a balance loss, alteration of metabolic pool and energy (Mancuso & Marras 2006). The more tolerant species allow the cells to avoid the severe ion imbalance by decreased membrane permeability for K^+ (Mancuso & Marras 2006).

Soil elemental toxicity is another factor which impacts on root K^+ homeostasis (Khabaz-Saberi & Rengel 2010; Shabala & Mackay 2011). Under these conditions, Mn and Fe will be available for the flooded root in the soil solution (Marschner 1995; Zeng et al. 2013) and H_2O_2, hydroxyl will be produced (Rodrigo-Moreno et al. 2013a). This results in a high amount of K^+ leaking from the cytosol, due to the ability of hydroxyl to activate ROS (reactive oxygen species) production while causing damage to the DNA and proteins, and impairing enzymatic activity (Rodrigo-Moreno et al. 2013a). Additionally, the K^+-selective channels (Demidchik et al. 2010) and non-selective (K^+ permeable) channels are activated (Zepeda-Jazo et al. 2011), facilitating K^+ loss by roots. External application of K^+ could successfully improve the adverse effects of flooding on plants, due, to the

K^+ increased plant growth, photosynthetic pigments and photosynthetic capacity, also improved plant nutrient uptake as a result of higher K^+, $Ca2^+$, N, $Mn2^+$ and $Fe2^+$ accumulation (Ashraf et al.2011).

Chapter 3 Effects of K^+ availability on growth and development of barley cultivars

3.1 Introduction

Worldwide, over 136 million tons of barley (*Hordeum vulgare* subsp. *vulgare* L.) is produced on about 56.6 million hectares, with an average yield of 2.4 t/ha (Gubatz 2010) Barley is a crucial crop and a significant source of food for the population in many countries (Fan & Saurkar 2006). Barley crops need K^+ as an essential nutrient for grain filling.

K^+ is an essential nutrient, accounting for up to 10% of plant dry weight (Leigh & Wyn Jones 1984; Watanabe et al. 2007). K^+ is important in crop metabolism and quality determination, while involved with many of the physiological processes supporting plant growth and development (Britto & Kronzucker 2008). K^+ also plays an essential role in energy transfer and utilization, protein synthesis, carbohydrate metabolism and the transport of sugars from leaves to fruits (Römheld & Kirkby 2010). As K^+ supply is insufficient to maintain seedling growth in most agricultural land, fertiliser is required to maximise the agricultural production (Römheld & Kirkby 2010). A deficiency of this element reduces both the number and size of leaves produced, leading to a reduction in the photosynthetic rate resulting in decreased yield and quality (Battie-Laclau et al. 2013; Huber 1984; Longstreth & Nobel 1980; Marschner 1995; Pettigrew 2008; Pleaslee & Moss 1968; Raschke & Goto 1975). Many studies have suggested photosynthetic capacities change with different K^+ supplies (Basile et al. 2003; Bednarz et al. 1998; Gerardeaux et al. 2009; Weng et al. 2007; Zhao 2001).

Plant requirements for K^+ change during the growing season; uptake is low when the plant is small, and increases during the late vegetative and flowering stages (Department of Agriculture and Food 2007; Trostle 2010). A deficiency of K^+ may result in poor root growth, restricted leaf development, fewer grains per head and smaller grain size, all of which affect both yield quantity and quality (Department of Agriculture and Food 2007). Even though K^+ concentrations are high in most agricultural soil, K^+ availability in soil solution is actually very low (around 10–100 µM),

because the K^+ supply to crop plants is a complex phenomenon involving the relationships of various K^+ fractions in the soil (Binjola et al. 2017; Lalitha 2014; White 2013).

The absorption of K^+ depends on many factors during the growing season, such as the amount of native soil K^+, the amount of fertilizer K^+ applied, the environmental conditions during the growing season and the management practices employed (Mengel et al. 1987; Naseem et al. 2014). K^+ supply from the soil is insufficient for economic crop production and hence K^+ fertilizer has to be supplied to crops for growth and yield. However, K^+ fertilizers are expensive, which makes it necessary to improve fertiliser management-practices or use genotypes with high potassium use efficiency (Fageria et al. 2011; White et al. 2010).

The ability of a plant root to take up K^+ from the soil is termed K^+ uptake efficiency (KUpE) and the ability of the plant to utilize the K^+ taken up to produce yield is termed as K^+ utilization efficiency (KUtE). Characteristics that increase KUpE in plants include:

1. exudation of organic acids that free K^+ bound in the soil;
2. high ability of roots to take up available K^+; and
3. rapid development of roots, high root/shoot ratio and high density of roots

Characteristics that increase KUtE include:

1. effective redistribution of K^+ within the plant;
2. ability to maintain water relations, photosynthesis and canopy cover;
3. high harvest index; and
4. tolerance of low concentrations of K^+ in tissues.

Factors that can contribute to the tolerance of low tissue K^+ include:

1. ability to maintain optimum K^+ in metabolically active cellular compartments when tissue K^+ concentration is low;
2. replacement of K^+ in some roles; and
3. redistribution of K^+ from senescent to younger tissues.

The first step in improving the K^+ use efficiency in crops would be to establish new genotypes with large root systems providing the plants with capacity to uptake K^+ more efficiently from native soil or from the applied fertiliser (Pettigrew 2008). The major objective of this chapter was

to investigate the impact of K^+ availability in soil on various agronomical characteristics of a broad range of barley genotypes to (1) identify genotypes with high- and low-K^+ requirements, (2) reveal the threshold of K^+ sensitivity; and (3) identify traits most dependent on K^+ supply.

3.2 Materials and Methods

3.2.1. Genetic material and experimental design

Thirty barley genotypes, originating from Australia, China, USA and Japan were evaluated for phenotypic variation in K^+ efficiency in shoot growth and grain yield (Table 3.1). The experiment was a randomized block design with 30 barley genotypes grown under four levels of K^+ (0.002 mM, 0.2 mM, 2 mM, and 20 mM) with six replicates. All treatments were replicated three times.

Seeds were surface sanitized in 10% commercial bleach available (sodium hypochlorite) (King White, Victoria, Australia) for 10 min, then thoroughly rinsed with distilled water. Ten seeds were planted in 6-inch pots. The plants were grown in coarse sand/vermiculate mix (70: 30 v/v) in a glasshouse (University of Tasmania, Hobart, Australia). The day/night temperatures were 20/10 °C with an average day length of 12 h. Plants were watered daily to excess, (i.e. run off), with Hoagland solution number 1 (Hoagland and Arnon 1950) that was modified using the method of Johnson et al. (1957) to allow manipulation of K^+ levels (Table 3.2). In order to control the potassium concentration of the nutrient solution, KNO_3 was replaced with $NaNO_3$, and KH_2PO_4 was replaced with $NH_4H_2PO_4$, in the same molar concentrations. Potassium was added to the nutrient solution using KCl at four different concentrations (0.002 mM, 0.2 mM, 2 mM, and 20 mM).

Table 3.1 Thirty genotypes of barley and their origin, maturity type and row type and sensitivity to salinity(S – spring; W – winter).

Genotype	Origin	Winter/spring type	Row type
YUQS	China	S	2
YYXT	China	W/S	2
ZP2	China	S	2
ZUG293	China	S	6
ZUG403	China	S	2
DYSYH	China	W	6
Gebeina	China	S	2
Yiwu Erleng	China	W	6
YSM1	China	S	2
YSM3	China	S	2
Yu6472	China	S	2
TX9425	China	S	2
Yan89110	China	S	2
Yan90260	China	S	2
RGZLL	China	W	6
Flagship	Australia	S	2
Keel	Australia	S	2
Schooner	Australia	S	2
Skiff	Australia	S	2
Dash	Australia	S	2
TF026	Australia	S	2
YF374	Australia	S	2
Franklin	Australia	S	2
Gairdner	Australia	S	2
Yerong	Australia	S	6
CM72	USA	S	6
Dayton	USA	W	6
Numar	USA	S	6
Kinu Nijo 6	Japan	S	2
Naso Nijo	Japan	S	2

Table 3.2 Composition of modified Hoagland solution used in the experiment.

Component	Concentration in stock solution	Volume (ml) of stock used in nutrient solution
$NaNO_3$	1 M	5
$Ca(NO_3)_2H_2O$	1 M	5
$MgSO_4$	1 M	2
$NH_4H_2PO_4$	1 M	1
Fe-EDTA	15 g/L	1
H_3BO_3	2.86 g/L	1
$MnCl_2H_2O$	1.81 g/L	1
$ZnSO_4.7H_2O$	0.22 g/L	1
$CuSO_4.5H_2O$	0.08 g/L	1
$H_2MoO_4.H_2O$	0.02 g/L	1

The pH of the modified Hoagland's solution was adjusted to 6.0 with 1M HCl. To reduce evaporation and increase germination, the soil surface was covered after sowing with a plastic sheet. Seven days after sowing, the plants were thinned to leave six uniform plants per plot.

3.2.2 Morphological measurements at maturity

At harvest, the number of tillers were counted and the height of each plant was measured from the ground to the top of the flower spike, not including the awns. The number of flower spikes was also recorded. Dry shoot weight was determined by taking all plant parts above ground, except for the spike, and drying at 60°C for 48 hours before taking the weight. Grain weight was measured after cleaning the grains, following which the seeds were counted using a Contador seed counter (CE Pfeuffer, Baumann Saatzuchtbedarf, Waldenburg, Germany).

3.2.3 Statistical analysis

The data was subjected to correlation analysis and analysis of variance using IBM SPSS Statistics. Average data for plant height, dry shoot weight, tiller number, spike number, grain number and grain weight for each of the four K^+ treatments were grouped (G#) using a hierarchical cluster analysis (HCA) based on Euclidean distances as a measure of dissimilarity and Ward's method as a clustering algorithm using XLSTAT software (Addinsoft, New York, NY, USA).

3.3 Results

3.3.1 Shoot dry weight

K^+ availability had a dramatic effect on plant dry shoot weight (Table 3.3; Figure 3.6A). When plants were grown at the lowest (0.002 mM) K^+ level, shoot dry weight did not exceed 2 g per plant. A 4-fold increase to (8 g) under 20 mM K^+ availability had a beneficial effect on plant performance, with some genotypes having a shoot dry weight of 5 g/plant. Interestingly, a further increase in K^+ availability did not improve shoot dry weight. At 2 mM, shoot dry weight was capped at 6 g/plant, while the best performing plants at 20 mM treatment had a shoot dry weight not exceeding 8 g/plant (Table 3.3).

Within each treatment, barley genotypes showed a broad range of variability in response to K^+ supplementation. At the lowest K^+ supply (0.002 mM) plant dry shoot weight ranged from highest 1.66 ± 0.06 g/plant in cv Franklin to lowest 0.21 ± 0.01 in cv Yu6472 (Table 3.3); an 8-fold difference in shoot dry weight. In addition to Franklin, the best performing genotypes were DYSYH and RG2LL, followed by Gairdner, Gebeina and YYXT. An increase in K^+ supply led to an increase in shoot dry weight in all genotypes, although the extent of their response differed significantly between genotypes. Franklin, DYSYH and YYXT also showed the greatest shoot dry weight values for all K^+ treatments.

Table 3.3 Genotypic variability in shoot dry weight (g/plant) of barley to K^+ supply values are mean ± SE (n = 6), (#G = group number). Genotypes have been divided into three groups according to cluster analysis (see section 3.2.3 and Figure 3.5).

		K^+ treatment (mM) average			
	Genotype	0.002	0.02	2	20
G#1	ZUG403	0.28±0.00	2.18±0.15	2.63±0.04	2.43±0.08
	YUQS	0.25±0.02	2.67±0.03	4.99±0.36	4.43±0.16
	Keel	0.23±0.02	0.89±0.09	1.49±0.16	1.54±0.03
	YSM1	0.49±0.08	1.58±0.10	2.11±0.03	1.85±0.10
	YSM3	0.33±0.03	1.76±0.13	2.28±0.27	2.21±0.24
	ZP2	0.28±0.03	2.94±0.23	2.65±0.35	2.54±0.63
	Flagship	0.49±0.03	1.67±0.13	2.27±0.60	2.99±0.13
	Dash	0.78±0.17	2.18±0.01	2.76±0.28	3.54±0.19
	Gebeina	1.28±0.02	3.16±0.40	3.93±0.25	3.43±0.09
G#2	Skiff	0.37±0.03	1.44±0.08	2.51±0.26	2.24±0.19
	ZUG293	0.85±0.05	3.98±0.00	3.68±0.16	4.17±0.00
	Gairdner	1.13±0.04	3.81±0.39	4.15±0.45	5.24±0.13
	Yerong	0.26±0.06	2.01±0.18	3.09±0.29	2.23±0.27
	Schooner	0.39±0.04	1.84±0.11	2.89±0.18	3.04±0.44
	YF374	0.41±0.01	1.38±0.28	1.85±0.08	1.93±0.04
	CM72	0.58±0.09	2.73±0.49	3.93±0.07	3.03±0.03
G#3	RGZLL	1.17±0.35	0.98±0.13	1.59±0.43	2.20±0.18
	Yan90260	0.31±0.03	0.77±0.00	0.94±0.18	0.92±0.20
	Yiwu Erleng	0.78±0.21	2.87±0.50	4.62±0.50	4.72±0.13
	Dayton	0.93±0.17	3.68±0.52	4.73±0.53	5.25±0.00
	DYSYH	1.25±0.50	4.94±0.24	5.92±0.78	8.02±0.38
	Yan89110	0.32±0.00	1.36±0.08	2.08±0.13	2.28±0.04
	Numar	0.38±0.03	1.08±0.03	1.53±0.06	2.16±1.03
	Yu 6472	0.21±0.01	1.18±0.20	1.55±0.20	1.38±0.00
	Naso Nijo	0.25±0.02	0.53±0.12	0.68±0.16	0.61±0.01
	TX9425	0.26±0.01	1.25±0.02	1.88±0.09	1.72±0.08
	TF026	0.24±0.06	0.93±0.05	1.78±0.03	1.54±0.13
	Kinu Nijo 6	0.24±0.01	0.52±0.05	0.69±0.20	0.69±0.10
	Franklin	1.66±0.06	4.46±0.46	4.99±0.08	7.56±0.99
	YYXT	1.03±0.02	4.80±0.17	5.18±0.30	5.05±0.00

3.3.2 Plant height

Plant height was significantly affected by K^+ availability (Table 3.4; Figure 3.6B). The largest difference in plant height among genotypes was observed between the lowest (0.002 mM) K^+ level and 0.02 mM treatments; a further increase in K^+ availability did not result in a major beneficial effect on plant height (Table 3.4). This result indicates that K^+ application above 0.02 mM K^+ did not increase plant height in barley and 0.02 mM K^+ would be the threshold of deficiency for plant height. The greatest variability between genotypes was shown for the 0.002 mM K^+ treatment. An increase in K^+ supply led to an increase in plant height in all genotypes, although the extent of the response differed significantly between genotypes. TX9425 under 20 mM K^+ treatment showed a 6-fold increase in the height it obtained under 0.002 mM, but some genotypes, (such as Franklin and Gairdner), showed only a 2-fold change across treatments. The contrasts in relative height increases of different genotypes demonstrates differing responsiveness to K^+ availability. The varieties that were most responsive to K^+ were TX9425, RGZLL and TF026.

Under the strongest K^+ treatment the tallest variety was 1.8 times the height of the shortest, while at the lowest K^+ supply (0.002 mM), plant height ranged from 11.4 ± 5.1 cm in cv TX9425 to 42.6 ± 0.6 cm in Gairdner; almost a 4-fold difference in plant height. The relatively low differences between genotypes under high K^+ treatment and the large differences under K^+ deficiency demonstrates the differences in genotypic responses to K^+, and the differing abilities to maintain growth when K^+ is in short supply. The varieties that under K^+ deficiency were closest to their heights under K^+ abundance were Keel, ZP2, Naso Nijo, Gairdner, and Franklin.

Table 3.4 Genotypic variability in height (cm/plant) of plants grown under various K^+ supply. Data are mean ± SEM (n = 6). Genotypes have been divided into three group according to a cluster analysis (see section 3.2.3 and Figure 3.5).

		K^+ treatment (mM) average			
	Genotype	0.002	0.02	2	20
G#1	ZUG403	31.2±1.9	81.3±0.0	85.1±3.8	68.6±0.0
	YUQS	27.9±0.0	61.6±8.3	66.0±0.0	53.3±2.5
	Keel	33.7±4.5	66.0±7.6	58.4±12.	61.0±5.1
	YSM1	31.8±1.3	74.9±3.8	58.4±10.2	58.4±0.0
	YSM3	25.4±1.3	69.9±3.8	78.7±10.4	54.6±1.3
	ZP2	34.9±0.6	69.9±1.3	61.6±1.9	67.3±1.3
	Flagship	36.2±0.6	73.7±0.0	77.5±6.4	69.9±6.4
	Dash	25.4±1.3	75.6±0.6	91.4±2.5	76.2±10.2
	Gebeina	36.2±3.2	75.6±7.0	88.9±2.5	72.4±3.8
G#2	Skiff	40.0±1.9	77.5±3.8	81.3±5.1	66.0±7.6
	ZUG293	31.2±1.9	81.3±0.0	85.1±3.8	68.6±0.0
	Gairdner	42.6±0.6	72.4±6.4	77.5±6.4	85.1±3.8
	Yerong	37.5±3.2	76.2±10.2	96.5±7.6	73.7±0.0
	Schooner	35.6±0.0	85.1±3.8	90.8±5.7	86.4±5.1
	YF374	36.8±1.3	77.5±3.8	89.5±5.7	86.4±5.1
	CM72	38.7±3.2	90.2±3.8	80.0±1.3	64.8±1.3
G#3	RGZLL	22.9±0.0	75.6±0.6	69.9±6.4	86.4±2.6
	Yan90260	36.2±0.6	71.1±0.0	80.0±1.3	74.9±3.8
	Yiwu Erleng	29.2±3.8	78.7±5.1	67.3±14.0	74.3±9.5
	Dayton	19.1±1.3	85.1±1.3	67.4±3.8	67.3±14.0
	DYSYH	26.7±1.3	60.3±26.0	92.7±1.3	78.7±10.2
	Yan89110	36.2±0.6	92.7±1.3	88.9±2.5	97.8±8.9
	Numar	33.7±0.6	67.9±7.0	69.9±1.3	76.2±12.7
	Yu 6472	27.9±0.0	68.6±7.6	62.3±1.3	53.3±2.5
	Naso Nijo	33.7±0.6	57.8±7.0	66.7±1.9	57.2±1.3
	TX9425	11.4±5.1	69.8±3.8	72.4±1.3	68.6±0.0
	TF026	15.9±4.5	72.4±1.3	76.2±2.5	72.4±3.8
	Kinu Nijo 6	29.2±1.3	61.0±0.0	69.9±8.9	62.2±1.3
	Franklin	38.7±8.3	59.7±6.4	59.7±8.9	77.5±11.4
	YYXT	22.9±2.5	69.9±1.3	77.5±1.3	78.7±0.0

3.3.3 Tiller number per plant

The tiller number was affected by K^+ availability (Table 3.5; Figure 3.6C). Genotypes exhibited significant increase in the tiller number when the K^+ level changed from 0.002 mM to 0.02 mM. However, further increase in K^+ from 0.02mM to 20 mM did not have any notable impact on the tiller number.

For most genotypes the 0.02 mM K^+ treatment had a major beneficial effect on tiller number (Table 3.5), but some genotypes such as RGZLL, Kinu Nijo 6, Yan89110, YU 6472, TF026, YSM1 and YSM3 did not respond to increased K^+ availability in the soil. The results indicate that further increases in K^+ availability did not increase tiller number in barley and 0.02 mM K^+ would be the threshold of deficiency for the tiller number for most genotypes.

While an increase in K^+ supply led to an increase in tiller number, this effect differed between genotypes. For example, genotype ZUG293 showed a 3-fold increase in the tiller number when the K^+ supply was increased from 0.002 to 0.02 mM but YSMI, RGZLL, Kinu Nijo 6 and Naso Nijo did not show a significant change across different K^+ supplementation (Table 3.5). At the lowest K^+ supply (0.002 mM) tiller number ranged from the lowest 1.3 ± 0.3 in cv Yerong to highest 4.4 ± 0.1 in cv Flagship (Table 3.5), around a 3-fold difference in the tiller number. In addition to the best performing genotypes were Flagship, Gebeina, Skiff and Schooner. Genotypes YYXT, ZP2, ZUG293, ZUG403, Franklin, and Dayton were the most responsive genotypes (showed more than a 2-fold change) in response to K^+ availability.

Table 3.5 Genotypic variability in tiller number of plants grown under various K^+ supply. Data are mean ± SEM (n = 6). Genotypes have been divided into three group according to a cluster analysis (see section 3.2.3 and Figure 3.5).

		K^+ treatment (mM) average			
	Genotype	0.002	0.02	2	20
G#1	ZUG403	2.0±0.0	4.8±1.5	5.8±0.1	3.8±0.5
	YUQS	2.5±0.3	4.4±0.8	4.9±0.3	4.1±0.4
	Keel	2.8±0.1	2.3±0.6	3.8±0.3	3.9±0.9
	YSM1	3.5±0.2	3.3±0.8	3.2±0.2	3.0±0.5
	YSM3	2.6±0.3	3.4±0.1	3.5±0.3	3.6±0.4
	ZP2	2.3±0.3	4.8±0.3	4.5±0.3	3.8±0.2
	Flagship	4.4±0.1	3.3±0.6	3.7±0.8	2.5±1.5
	Dash	2.1±0.1	3.1±0.6	4.1±0.1	4.3±0.3
	Gebeina	4.1±0.5	6.3±1.3	6.4±0.6	2.7±0.1
G#2	Skiff	3.6±0.3	5.2±0.5	6.3±0.0	4.4±0.6
	ZUG293	1.7±0.2	4.7±0.0	5.0±0.3	7.7±0.0
	Gairdner	3.3±0.3	5.7±0.0	6.1±0.6	4.5±0.2
	Yerong	1.3±0.3	2.2±0.2	4.4±0.4	2.4±0.3
	Schooner	3.6±0.3	5.2±0.8	5.3±0.9	5.7±0.8
	YF374	2.3±0.6	4.3±1.3	4.3±0.8	4.1±0.9
	CM72	2.5±0.5	4.2±0.8	6.4±0.3	3.7±0.2
G#3	RGZLL	2.1±0.4	2.3±0.3	2.8±0.0	3.3±0.2
	Yan90260	2.7±0.0	2.9±0.1	3.4±1.3	1.5±0.3
	Yiwu Erleng	2.8±0.8	3.3±0.1	4.8±0.5	3.8±0.3
	Dayton	2.6±0.1	5.3±0.3	4.8±0.9	3.0±0.3
	DYSYH	3.6±0.1	6.5±0.3	5.3±1.4	5.9±0.6
	Yan89110	3.2±0.2	2.8±0.3	4.5±0.2	2.7±0.2
	Numar	2.2±0.3	4.2±0.2	4.5±0.3	5.3±0.6
	Yu 6472	2.1±0.3	1.8±0.7	3.3±0.4	2.3±0.4
	Naso Nijo	3.1±0.1	2.4±0.4	1.8±0.3	1.8±0.4
	TX9425	2.3±0.2	3.6±0.1	4.4±0.1	2.4±0.6
	TF026	2.6±1.1	2.8±0.5	4.2±0.8	2.7±0.7
	Kinu Nijo 6	2.3±0.1	2.2±0.3	2.0±0.2	2.2±0.2
	Franklin	3.0±0.2	6.3±0.3	7.8±0.3	8.3±0.3
	YYXT	2.3±0.2	4.8±0.2	6.4±0.3	6.8±0.0

3.3.4 Grain weight per plant

Plant grain weight was also affected by K^+ availability (Table 3.6; Figure 3.6D). Most genotypes showed the highest grain weight difference between the lowest (0.002 mM) K^+ level and 0.02 mM, but a further increase in K^+ availability did not result in a major beneficial effect on grain weight in most genotypes. For most genotypes, the 0.02 mM K^+ treatment had a major beneficial effect on grain weight (Table 3.6) but some genotypes like Kinu Nijo 6 and Naso Nijo did not respond to increased K^+ availability in the soil. As most genotypes did not have a statistically significant increase in grain yield between the 0.02 mM and 2 mM treatments, 0.02 mM K^+ could be considered the threshold of deficiency for grain weight for most genotypes. The highest variability between genotypes was shown in the 0.002 mM potassium treatment, where genotypes Dayton, DYSYH and RGZLL did not produce any grain.

Although it should be noted that not all differences were statistically significant, thirteen genotypes (i.e. 43%) had their maximum grain yield in the 20 mM treatment, 10 genotypes, (30%) had their greatest yield under 2 mM, and three, (10%), reached their maximum with only 0.02 mM K^+ fertilisation. Another 3 genotypes, (10%), had essentially identical yields in the 0.02 and 2 mM treatments, and one genotype (Franklin), showed no reliable differences in yield across all treatments.

An increase in K^+ supply led to an increase in grain weight in most genotypes, although the extent of their response differed significantly between genotypes. Gairdner showed 17-fold change in from 0.002 to 0.02 mM K^+ supplementation but some genotypes did not show a significant change across different K^+ supplementation (Table 3.6). At the lowest K^+ supply (0.002 mM) the genotypes Dayton, DYSYH and RGZLL did not produce grain. For the genotypes producing grain at 0.002 mM, the grain weight ranged from the lowest 0.03±0.01 g/plant in Gairdner and YYXT to the highest of 0.40 ± 0.04 in cv Skiff and YF374 (Table 3.6); a 40-fold difference in grain weight. The best performing genotypes were Skiff, YF374 and Flagship. Gebeina was the most responsive genotype (showed a 19-fold change) in response to K^+ availability.

Table 3.6 Genotypic variability in grain weight (g/plant) of plants grown under various K^+ supply. Data are mean ± SEM (n = 6). Genotypes have been divided into three group according to a cluster analysis (see section 3.2.3 and Figure 3.5).

		K^+ treatment (mM) average			
	Genotype	0.002	0.02	2	20
G#1	ZUG403	0.20±0.01	1.05±0.03	1.20±0.20	1.03±0.05
	YUQS	0.20±0.01	1.05±0.03	1.20±0.20	1.03±0.05
	Keel	0.25±0.02	0.38±0.08	0.93±0.19	0.78±0.06
	YSM1	0.06±0.02	0.62±0.10	0.67±0.07	0.92±0.01
	YSM3	0.26±0.06	0.94±0.15	1.22±0.11	0.65±0.11
	ZP2	0.28±0.03	1.21±0.05	1.09±0.06	1.18±0.03
	Flagship	0.38±0.09	0.69±0.04	0.75±0.10	0.97±0.29
	Dash	0.16±0.05	0.91±0.10	0.92±0.16	0.67±0.03
	Gebeina	0.06±0.01	1.13±0.04	1.20±0.13	1.48±0.11
G#2	Skiff	0.40±0.05	0.43±0.05	1.15±0.08	0.88±0.06
	ZUG293	0.14±0.01	0.92±0.00	0.98±0.05	1.08±0.00
	Gairdner	0.03±0.01	0.52±0.06	0.48±0.26	0.61±0.28
	Yerong	0.34±0.01	0.90±0.14	0.93±0.06	1.15±0.05
	Schooner	0.32±0.03	0.93±0.16	1.02±0.57	0.76±0.03
	YF374	0.40±0.04	0.55±0.26	0.93±0.07	0.55±0.02
	CM72	0.28±0.07	0.59±0.07	0.56±0.11	1.43±0.04
G#3	RGZLL	0.00±0.00	0.14±0.00	0.22±0.12	0.33±0.15
	Yan90260	0.15±0.00	0.27±0.05	0.43±0.11	0.34±0.04
	Yiwu Erleng	0.04±0.04	0.54±0.01	0.16±0.15	0.24±0.07
	Dayton	0.00±0.00	0.00±0.00	0.35±0.03	0.41±0.00
	DYSYH	0.00±0.00	0.07±0.00	0.16±0.11	0.75±0.04
	Yan89110	0.12±0.03	0.49±0.05	0.47±0.08	0.31±0.16
	Numar	0.20±0.00	0.45±0.01	0.39±0.30	0.74±0.16
	Yu 6472	0.18±0.01	0.61±0.10	0.61±0.25	0.43±0.11
	Naso Nijo	0.21±0.02	0.28±0.05	0.40±0.14	0.26±0.00
	TX9425	0.06±0.03	0.33±0.05	0.20±0.06	0.41±0.05
	TF026	0.18±0.03	0.32±0.01	0.17±0.16	0.19±0.12
	Kinu Nijo 6	0.20±0.02	0.27±0.03	0.29±0.10	0.16±0.00
	Franklin	0.03±0.03	0.04±0.01	0.00±0.00	0.06±0.01
	YYXT	0.03±0.02	0.00±0.00	0.14±0.01	0.38±0.00

3.3.5 Grain number per plant

Grain number showed the same response as grain weight in response to increased K^+ availability in the soil (Table 3.7; Fig 3.6E). Most of the genotypes showed the highest grain number difference between the lowest 0.002 mM and 0.02 mM K^+, but a further increase in K^+ availability did not result in a significant beneficial effect on grain number in most genotypes. For most genotypes 0.02 mM K^+ treatment had a major beneficial effect on grain number (Table 3.7), but some genotypes like Kinu Nijo 6 and Naso Nijo did not show any significant response to an increase in K^+ availability in the soil. The result indicates that 0.02 mM K^+ would be the threshold of deficiency for grain number for barley. The highest variability between genotypes was shown in the 0.002 mM K^+ treatment. At 0.002 mM, genotypes Dayton, DYSYH and RGZLL did not produce any grain under a K^+ deficiency.

While the increase in K^+ supply led to an increase in grain number, the extent of their response differed significantly between genotypes. Gebeina showed a 21-fold change in comparison of 0.002 and 0.02 mM K^+ supplementation but some genotypes (e.g. Franklin, TF026, Kinu Nijo 6) did not show a significant change across different K^+ supplementation (Table 3.7).

At the lowest treatment (0.002mM) the plant grain number per plant ranged from 0.9 ± 0.9 in Franklin to the highest at 12.1 ± 2.3 in Flagship (Table 3.7); the 12-fold difference in grain number. The genotypes Flagship, Skiff and YF374 produced the highest grain numbers at a low K^+ treatment. Gebeina was the most responsive genotype (showed a 21-fold change) in response to K^+ availability.

Table 3.7 Genotypic variability in grain number of plants grown under various K^+ supply. Data are mean ± SEM (n = 6). Genotypes have been divided into three group according to a cluster analysis (see section 3.2.3 and Figure 3.5).

		K^+ treatment (mM) average			
	Genotype	0.002	0.02	2	20
G#1	ZUG403	6.2±0.0	23.2±3.0	30.0±1.7	38.5±8.0
	YUQS	6.6±0.2	26.6±2.4	34.1±3.1	36.7±0.1
	Keel	9.6±0.3	15.4±3.3	27.9±0.6	38.6±4.6
	YSM1	1.8±0.6	28.4±4.8	25.8±3.3	43.4±3.6
	YSM3	9.2±0.8	29.9±2.9	37.2±5.6	32.8±5.5
	ZP2	9.8±1.7	34.3±0.8	35.5±3.0	27.3±1.4
	Flagship	12.1±2.3	26.3±0.2	32.1±3.8	41.4±12.9
	Dash	9.2±3.0	39.6±0.3	42.5±5.8	41.6±2.4
	Gebeina	1.9±0.5	40.1±1.3	36.3±4.9	55.6±4.4
G#2	Skiff	11.5±59.7	15.0±1.7	41.2±10.8	27.8±0.8
	ZUG293	7.2±1.8	29.3±0.0	28.1±1.1	32.3±0.0
	Gairdner	1.7±0.8	21.8±1.6	18.2±9.7	49.2±3.8
	Yerong	7.1±0.4	29.5±2.8	24.2±0.3	37.3±5.3
	Schooner	9.3±0.8	29.4±3.4	34.2±2	36.8±0.3
	YF374	10.4±0.6	16.1±8.6	21.8±2.3	19.3±0.3
	CM72	7.0±0.8	12.2±0.3	11.7±2.5	41.5±0.2
G#3	RGZLL	0.0±0.0	4.3±2.2	8.7±2.0	20.7±7.7
	Yan90260	4.8±0.1	9.2±1.3	11.4±3.6	14.7±3.2
	Yiwu Erleng	1.2±1.2	20.6±1.1	7.3±6.9	7.8±1.3
	Dayton	0.0±0.0	0.0±0.0	18.1±4.1	16.6±1.9
	DYSYH	0.0±0.0	6.1±0.3	12.3±0.6	30.0±0.0
	Yan89110	5.5±0.7	11.8±1.8	11.8±0.9	12.6±6.8
	Numar	5.8±0.5	12.6±0.9	14.2±4.2	17.1±0.9
	Yu 6472	5.5±0.5	21.7±1.7	15.0±5.0	13.8±3.8
	Naso Nijo	7.7±0.5	9.3±1.1	12.8±3.0	12.9±1.6
	TX9425	2.4±0.8	16.4±4.6	8.8±4.7	17.1±3.4
	TF026	9.83±0.5	15±1.7	7.58±7.3	9.83±6.5
	Kinu Nijo 6	6.7±0.5	10.9±0.8	9.3±2.1	8.2±0.7
	Franklin	0.9±0.9	1.4±0.4	0.0±0.0	2.7±0.2
	YYXT	1.5±0.8	0.0±0.0	6.5±1.5	12.5±0.0

3.3.6 Spike number per plant

Spike number per plant showed the same response as grain number to increased K^+ availability in the soil (Table:3.8; Figure 3.6F). Most genotypes showed the highest spike number difference between the lowest level (0.002 and 0.02 mM) potassium, but a further increase in K^+ availability did not result in a significant beneficial effect on spike number in most genotypes. For most genotypes, the 0.02 mM K^+ treatment had a major beneficial effect on spike number (Table 3.8) but some genotypes like Kinu Nijo 6 and Naso Nijo did not show a significant response to increased K^+ availability in the soil. These results indicate K^+ availability did not increase spike number in barley and 0.02 mM K^+ would be the threshold of deficiency for spike number. The highest variability between genotypes was shown in the 0.002 mM potassium treatment, where the genotypes Dayton, DYSYH and RGZLL did not produce any grain under a K^+ deficiency. While the increase in K^+ supply led to an increase in spike number, the extent of responses differed significantly between genotypes. The genotype Gebeina showed a 21-fold change in comparison to 0.002 and 0.02 mM K^+ supplementation, but some genotypes (e.g. Yerong, Yiwu Erleng and Kinu Nijo 6) did not show a significant change across different K^+ supplementation (Table 3.8).

At lowest the K^+ supply (0.002 mM) plant spike number ranged from the lowest of 0.5 ± 0.0 in Franklin to the highest 12.1 ± 2.3 in Flagship (Table 3.8); a 12-fold difference in spike number. The best performing varieties were Flagship, Skiff and YF374. Gebeina was the most responsive genotype (showed the 21-fold change) in response to K^+ availability.

Table 3.8 Genotypic variability in spike number of plants grown under various K^+ supply. Data are mean ± SEM (n = 6). Genotypes were divided into three group according to a cluster analysis (see section 3.2.3 and Figure 3.5).

		K^+ treatment (mM) average			
	Genotype	0.002	0.02	2	20
G#1	ZUG403	1.7±0.3	4.9±1.4	5.5±0.2	4.3±0.3
	YUQS	1.2±0.8	4.4±0.6	2.9±0.2	3.6±0.3
	Keel	2.5±0	2.2±0.5	3.3±0.8	3.2±0.2
	YSM1	1.9±0.4	2.8±0.3	1.9±0.3	2.4±0.6
	YSM3	1.8±0.2	2.6±0.1	2.7±0.3	1.8±0.0
	ZP2	2.1±0.4	4.3±0.3	2.7±0.2	3.3±0.1
	Flagship	2.8±0.1	2.8±1.1	2.7±0.2	1.8±0.8
	Dash	1.1±0.1	2.1±0.3	2.0±0.0	1.4±0.1
	Gebeina	0.9±0.1	4.1±0.9	2.8±0.2	2±0.4.0
G#2	Skiff	2.9±0.1	2.5±0.2	3.4±0.6	2.5±0.2
	ZUG293	1.1±0.1	2.8±0.0	2.3±0.1	5.2±0.0
	Gairdner	1.2±0.0	1.8±0.3	2.3±0.1	1.9±0.3
	Yerong	1.7±0.2	1.8±0.1	1.6±0.1	1.4±0.1
	Schooner	2.3±0.2	1.8±0.3	2.3±0.3	2.2±0.0
	YF374	2.6±0.1	3.4±0.8	3.5±0.0	2.1±0.6
	CM72	1.8±0.3	2.5±0.5	3.1±0.4	3.9±0.3
G#3	RGZLL	0.0±0.0	0.8±0.1	1.6±0.1	1.3±0.3
	Yan90260	2.2±0.2	1.2±0.2	2.6±0.9	1.4±0.4
	Yiwu Erleng	1.2±1.2	1.8±0.2	1.0±0.7	0.7±0.3
	Dayton	0.0±0.0	0.8±0.1	1.1±0.1	1.9±0.1
	DYSYH	0.0±0.0	1.0±0.0	1.2±0.3	1.3±0.3
	Yan89110	2.0±0.5	1.6±0.1	2.8±0.4	1.3±0.1
	Numar	1.4±0.3	2.6±0.1	3.3±0.1	2.3±0.9
	Yu 6472	1.6±0.1	1.8±0.3	2.6±0.4	1.5±0.3
	Naso Nijo	2.3±0.2	1.7±0.5	1.6±0.1	1.3±0.0
	TX9425	1.8±0.7	2.4±0.3	3.2±0.2	1.5±0.5
	TF026	2.3±0.8	2.1±0.4	1.8±0.0	2.0±0.7
	Kinu Nijo 6	1.5±0.2	1.0±0.0	0.8±0.2	1.2±0.2
	Franklin	0.5±0.0	0.3±0.1	0.2±0.2	2.3±0.4
	YYXT	1.0±0.2	1.0±0.2	1.7±0.2	2.5±0.0

3.3.7 Correlation between components of yield

There was a significant positive correlation between grain yield and grain number. When analysing all genotypes within a treatment, a strong correlation was found between grain yield and grain number in all treatments, and the highest correlation was in the 0.02 mM treatment (R^2 = 0.86). Grain number was the most important yield trait because it had the highest correlation with grain yield (Figure 3.2A-D). Spike number was similarly correlated with yield, but the correlation coefficient was lower. The ranking of treatments for the strength of the correlation was in the order 0.02, 0.002, 2.0 and 20 mM (R^2 = 0.61, R^2 = 0.58, R^2 = 0.39 and R^2 = 0.36) (Figure 3.2E-H). Grain yield did not show a significant correlation with plant height (Figure 3.1E-H). Similarly, no correlation was found between grain yield and dry shoot weight (Figure 3.1A-D). The results also indicate increasing tiller numbers did not result in an increase in the number of fertile spikes or grain number. In addition, the results showed no correlation between grain yield and tiller number for all treatments (Figure 3.3A-D).

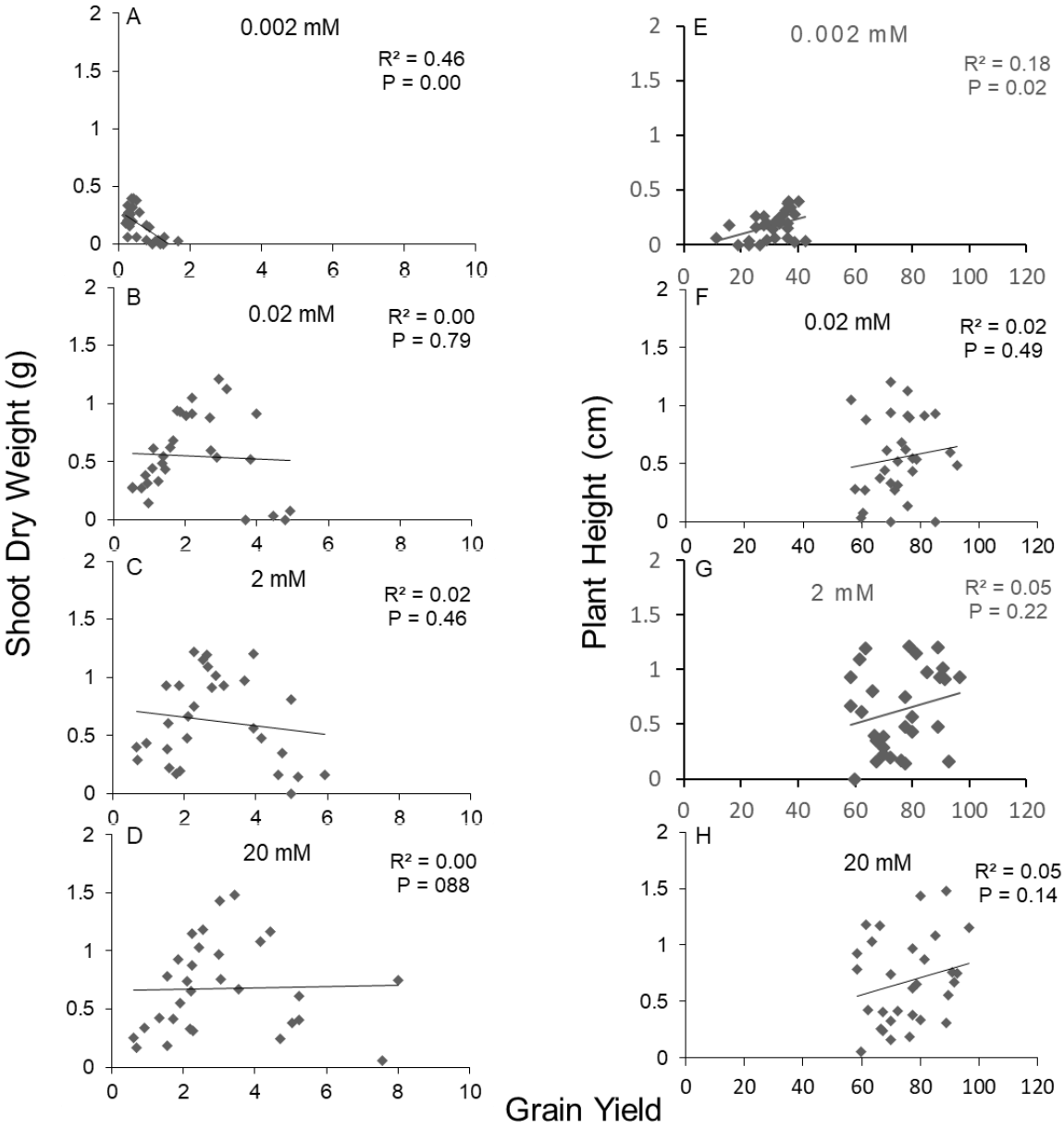

Figure 3.1 Correlation between grain yield and shoot dry weight (A, B, C, and D) and plant height (E, F, G and H) of 30 barley genotypes. Each point represents a separate genotype grown under various K^+ treatments: 0.002 mM (A and E), 0.02 mM (B and F), 2 mM (C and G), 20 mM (D and H) under glasshouse conditions.

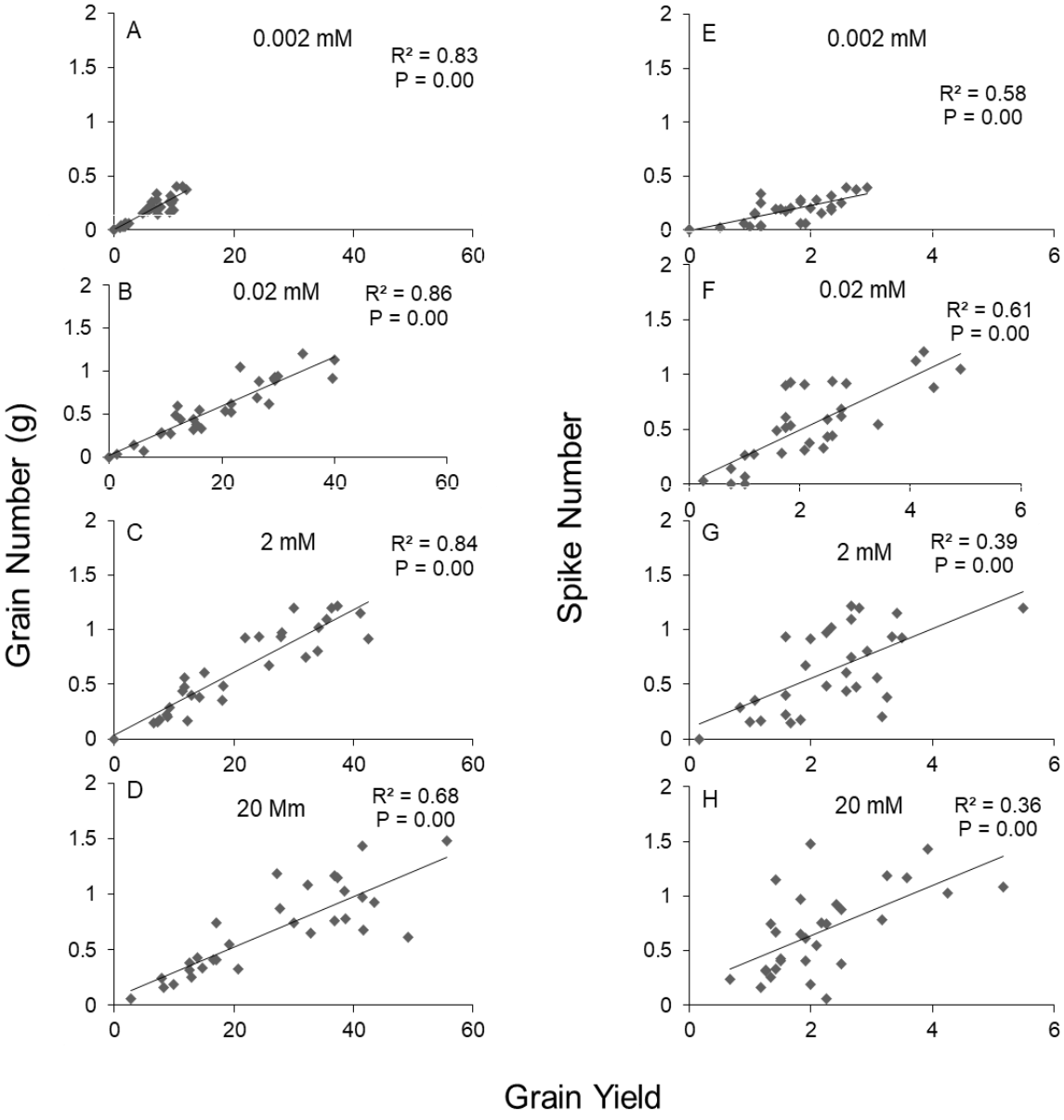

Figure 3.2 Correlation between grain (A, B, C, and D), and spike number (E, F, G and H) of 30 barley genotypes grown under four K^+ treatments 0.002 mM (A and E), 0.02mM (B and F), 2 mM (C and G), 20 mM (D and H) in glass house conditions. Grain Yield, grain number and spike number were measure at harvest stage.

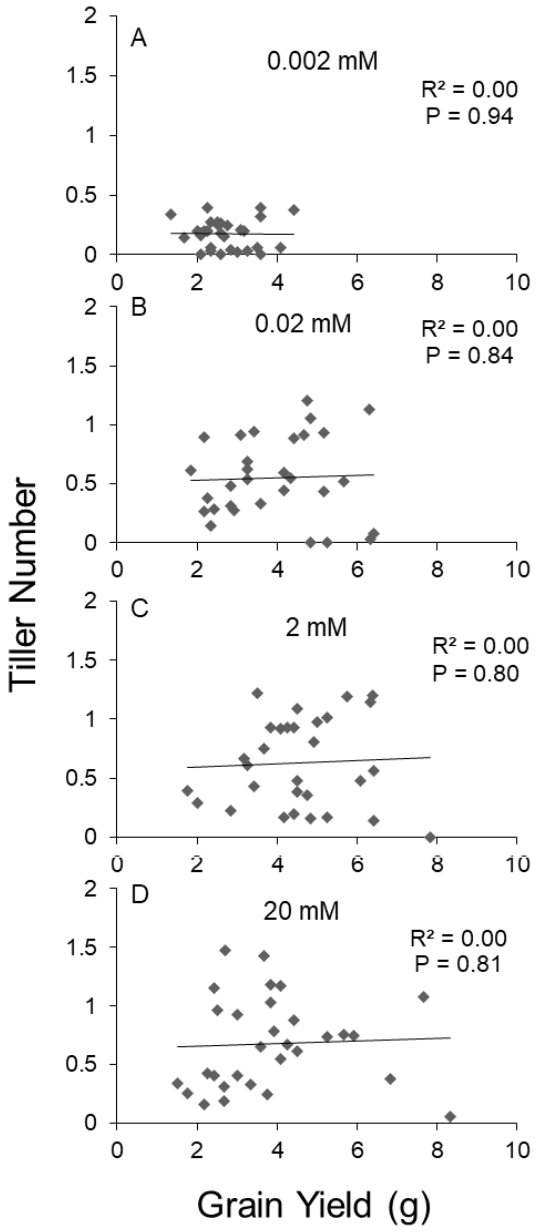

Figure 3.3 Correlation between grain yield and tiller number of 30 barley genotypes been grown under four K^+ treatments 0.002mM (A and E), 0.02mM (B and F), 2mM (C and G), 20mM (D and H) under glass house conditions. Spike number and Grain Yield measured at harvest stage.

3.3.8 Principle Component Analysis and Cluster analysis

Principal component analysis (PCA) based on all variables was used to discriminate between genotypes. The ordination analysis indicated the principal component axes AX1 and AX2 accounted for 42 % and 32% of the sums of squares, respectively (Figure 3.4 and 3.5). AX1 was mainly linked to spike number, while AX2 was influenced by tiller number and dry shoot weight.

The results of the cluster analysis are presented in Figure 5, and shows that the genotypes were classified into three groups based on agronomical traits. The first group (G1) was K^+-responsive and positive for AX1 (Figure 3.4). G1 contains nine genotypes including ZUG403, YUQS, Keel, YSMI, ZP2, Flagship, Dash, Gebeina and Skiff (Figure 3.4 and 3.5). These plants showed the highest values for grain weight, grain number and number of spikes. The second group (G2) was neutral for AX1 but positive for AX2 (Figure 3.4), and contained seven genotypes including ZUG293, Gairdner, Yerong, Schooner, YF374, CM72 and RGZLL. These plants were taller and had the greatest shoot dry weight and tiller number but had intermediate values for grain weight, number of grains and number of spikes. Thus, these were classified as moderately K^+ responsive. The third group (G3) was negative for AX1 (Figure 3.4) and contained 14 genotypes (YYXT, Dayton, DYSYH, Franklin, Yiwu Erleng, Yu6472, TF026, TX9425, Yan89110, Yan90260, Kinu Nijo 6, Naso Nijo, Numar and RGZLL). These genotypes had lowest shoot dry weight, were shorter, and had fewer tillers, grains, grain weight and number of spikes and were classified as unresponsive to K^+ fertilization.

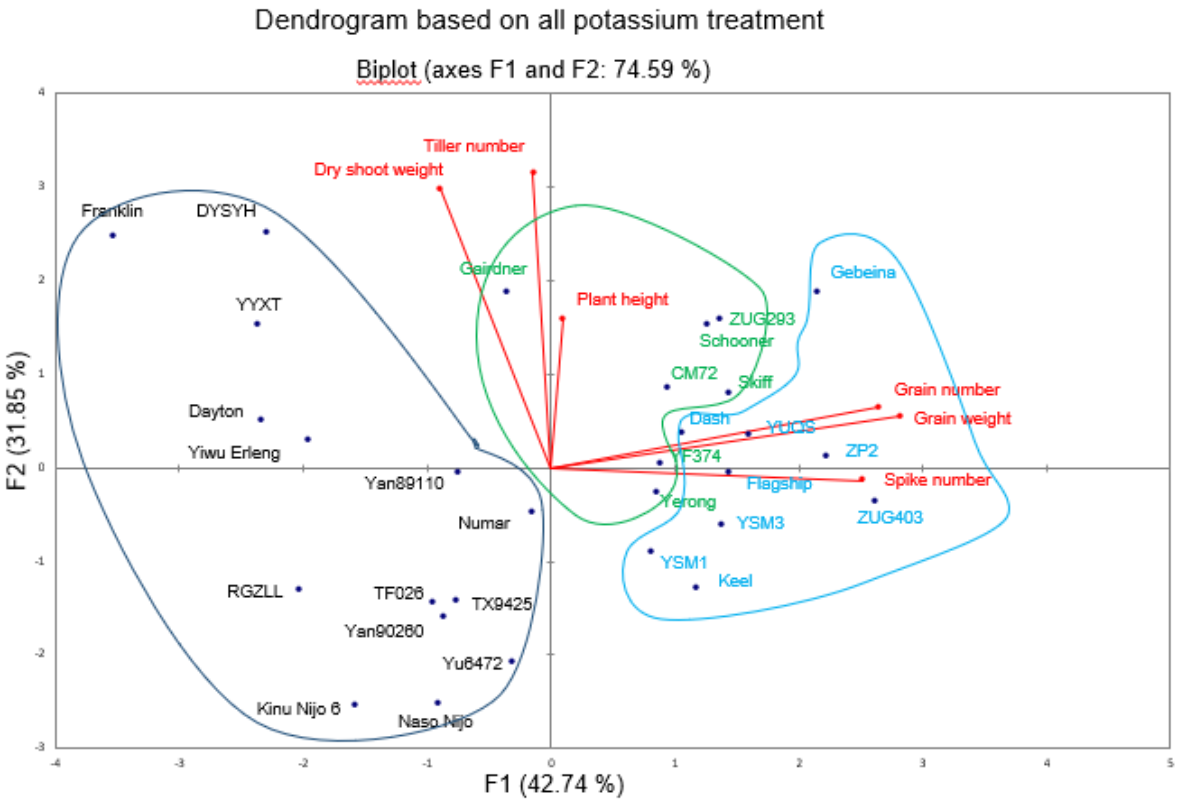

Figure 3.4 Principle component analysis for AX1 and AX2 for all data. The axes accounted for 74.8% of the sums of squares. Genotype groups correspond to the following colours: G#1, blue; G#2, green; and G#3.

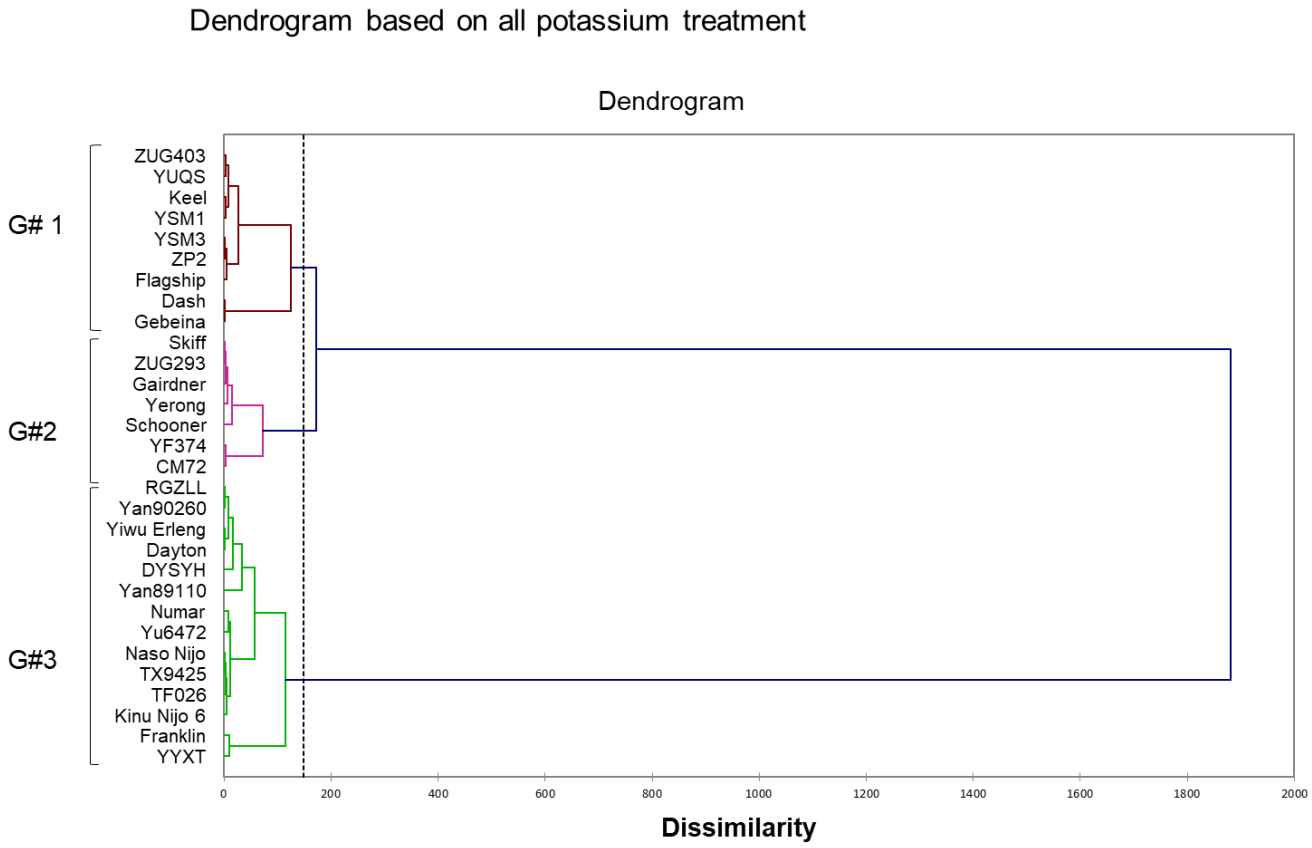

Figure 3.5 Genotype groups applied to all data. The dendrogram shows fusion levels at which the groups join. The vertical dashed line represents the truncation into three genotype groups using Ward's agglomerative clustering algorithm**.**

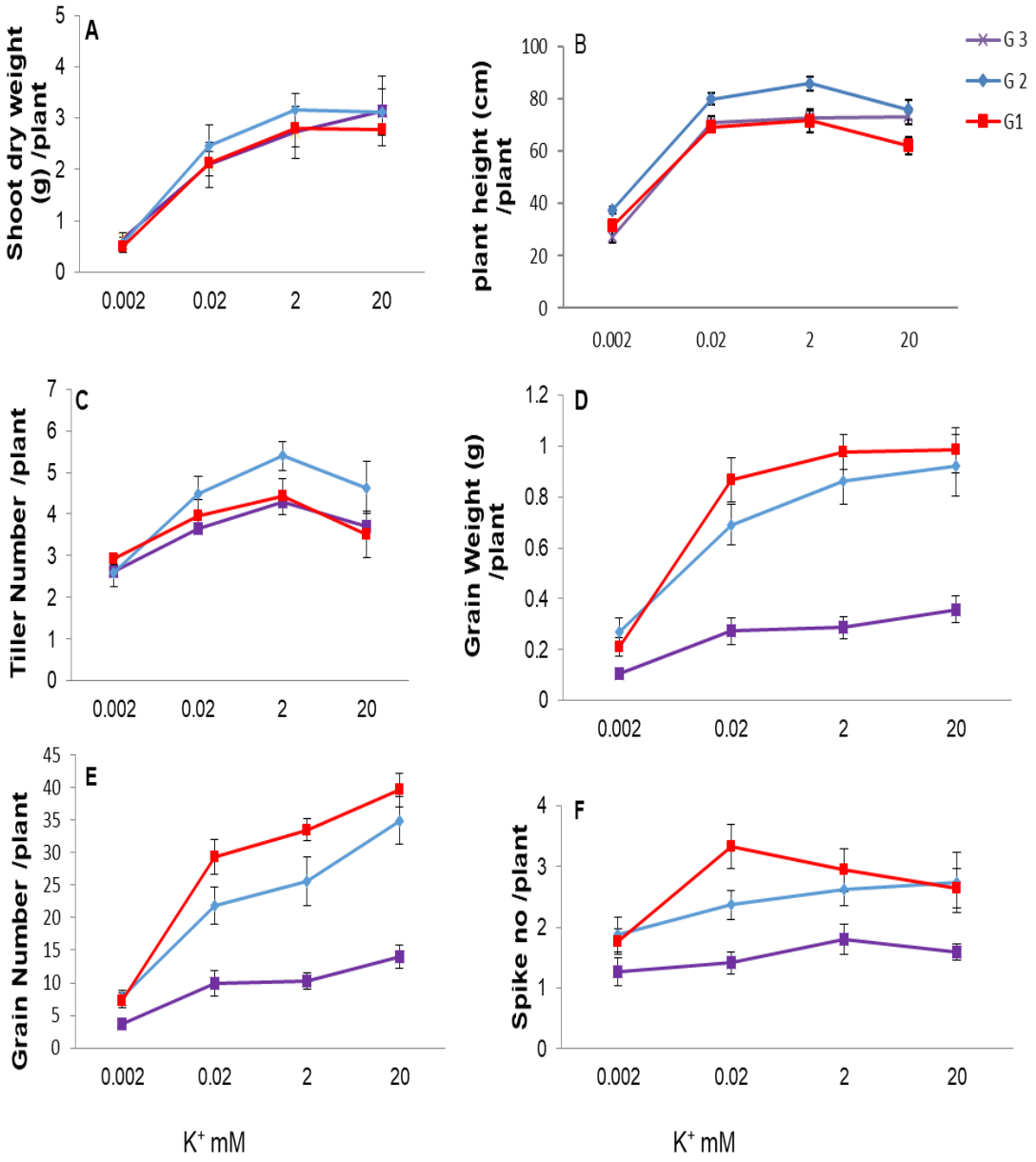

Figure 3.6 Comparison of the groups produced by cluster analysis, showing the differences between groups in A) shoot dry weight, B) plant height, C) tiller number, D) grain weight, E) grain number and F) spike number per plant, respectively, for the four K^+ treatments. Group one contains nine genotypes (ZUG403, YUQS, Keel, YSMI, ZP2, Flagship, Dash, Gebeina and Skiff), group two contains seven genotypes (ZUG293, Gairdner, Yerong, Schooner, YF374, CM72 and RGZLL), group three contains fourteen genotypes (YYXT, Dayton, DYSYH, Franklin, Yiwu Erleng, Yu6472, TF026, TX9425, Yan89110, Yan90260, Kinu Nijo 6, Naso Nijo, Numar and RGZLL).

3.4 Discussion

The objective of the screening experiment was to identify the genotypes responsive to K^+ fertilization and those tolerant of K^+ deficiency, and the agronomical traits that contributed to high yield under conditions of K^+ deficiency. K^+ is an essential macronutrient for plant growth and development. Developing crop cultivars with a high impact on K^+ use efficiency under deficient conditions can be an effective approach to overcome K^+ deficiency.

Plants were taller and there was an overall increase in grain yield in response to K^+ application, which was positively correlated with number of spikes and grain number per plant. According to Sweeney et al. (2000) and Sharma et al. (2005), an increase in grain yield with supplementation of K^+ was due to improved seed weight. Amanullah & Irfanullah (2016) similarly reported an increase in yield components and grain yield with the application of K^+. The increase in yield components with applied K^+ level might be due to efficient intake of K^+ under deficient conditions, where many genotypes showed their capacity to assign biomass production to grain yield. The differences in metabolic pathways result in differences in energy distribution and capacity of low K^+ stress adaptation.

Shoot dry weight wasn't correlated with grain yield when data from all genotypes were analysed together. These results are similar to Damon & Rengel (2007), George et al. (2002) and Heckman & Kamprath (1992) who reported that shoot dry weight of plants are not a good indicator of low K^+ efficiency at physiological maturity. In contrast, increased plant shoot dry weight with greater K^+ availability has been reported in the literature at the tillering stage (Yang et al. 2003) and linked to activation of enzymes, protein synthesis and increased uptake of other nutrients (Akhtar et al. 2003; Asif & Anwar 2007). In the current study, it would be expected that a correlation between shoot weight and grain yield would be weak or absent, because some of the genotypes in the study are normally short, even when in good conditions and yielding well, while others are relatively tall. However all genotypes had both plant height and shoot weight strongly suppressed by K^+ deficiency, leading to a pronounced relationship between grain yield and both plant height and shoot weight. Thus while plant height and shoot weight can not be directly compared between different genotypes, the relative changes in shoot weight and plant height are useful measures for discerning genotypes that are more or less limited by K^+ deficiency and which are more or less responsive to K^+ fertilisation.

Most genotypes reached their maximum shoot weight in the 2 mM K^+ treatment, and had little or no difference between this and the 20 mM K^+ treatment. There were some notable exceptions, including the variety Franklin increased from 4.99 g/plant under 2 mM treatment to 7.56 g/plant with 20 mM treatment. Franklin was thus responsive to a very high rate of fertiliser, which should be considered in the production of this variety.

Similarly, the maximum plant height for most genotypes was reached in the 2 mM K^+ treatment, and height in the 20 mM K^+ treatment was no different or sometimes considerably less. A small number of varieties (notably Franklin) continued to gain height significantly in response to the 20 mM treatment. Conversely a small number of varieties reached their maximum height in the 0.02 mM treatment.

Some genotypes had a close association between grain yield and plant height and/or shoot weight while in others the association was not so simple. For example, the variety Yiwu Erleng was significantly taller in the 0.02 mM treatment than in any others and also had its highest grain yield in this treatment, while in the variety CM72 treatments above 0.02 mM K^+ decreased plant height substantially even though grain yield showed a very large response to the 20 mM treatment. CM72 was one of the tallest varieties in the study, and it appears that excess K^+ that stressed the plant somewhat and suppressed height growth has a positive effect on partitioning resources in favour of grain production. A plausible explanation for this is that the 20 mM treatment induces sufficient osmotic stress to stimulate force the plant to divert resources away from height growth for producing compatible solutes instead. Unlike the structural carbohydrates that are made for attaining plant height, compatible solutes are easily translocated and are thus readily available for grain production. This raises the interesting prospect that high rates of potassium fertiliser could be used to increase yields of varieties that are desirable from the point of view of adaptation to their environment, but have their yield potential reduced by poor partitioning of resources because of excessive height. While investing energy in height is a sensible strategy for plants that must support a deep root system for drought tolerance (Mambani and Lal 1983), or compete with weeds, it is a waste of resources if weeds are well controlled and a deep root system isn't especially needed.

The results for plant height and shoot weight demonstrate the potential of K^+ administration to influence grain yield via effects on plant size and thus capacity to produce and store assimilates necessary for grain production. The results also show that while the majority of genotypes have similar K^+ needs, there are some that have very different needs, and won't reach their maximum

yield potential under a potassium fertilisation regime that suits the majority of varieties. It is thus desirable for potassium fertiliser recommendations to be tailored to varieties. The current study was not able to determine the optimum K^+ rate for any variety, because the wide span of K^+ treatments necessitated large intervals in treatment strength. Never the less, some genotypes showed rather small differences between adjacent treatments, thus allowing flexibility in fertilisation. For example the genotype Yan 89110 produced its greatest yield (0.49 g/plant) in the 0.02 mM treatment, but was very little different (0.47 g/plant) in the 2 mM treatment, despite 100 fold difference in treatment strength. PCA was used to identify three groups of genotypes where the yield and components of yield differed in response to K^+ deficiency conditions. The first group (G1) was K^+-responsive and positive for AX1 (Figure 3.4). G1 contains nine genotypes including ZUG403, YUQS, Keel, YSMI, ZP2, Flagship, Dash, Gebeina and Skiff (Figure 3.4 and 3.5). These plants showed the highest average values for grain weight, grain number and number of spikes. For example, in response to increasing K^+ treatment from 0.002 to 2 mM, the average grain weight in G1 increased from 0.21 g/plant to 0.97 g/plant (4.62 fold) whereas the next most responsive genotype group increased from 0.27 g/plant to 0.92 g/plant (3.41 fold).

The second group (G2) was neutral for AX1 but positive for AX2 (Figure 3.4), and contained seven genotypes including ZUG293, Gairdner, Yerong, Schooner, YF374, CM72 and RGZLL. These plants were taller and had the greatest shoot dry weight and tiller number but had intermediate values for grain weight, number of grains and number of spikes. Thus, these were classified as moderately K^+ responsive. For example, in response to increasing K^+ treatment from 0.002 mM to 20 mM, groups 1 and 3 increased their average grain numbers 5.35 and 3.79-fold respectively, while group 2 underwent an intermediate increase of 4.50 fold.

The third group (G3) was negative for AX1 (Figure 3.4) and contained 14 genotypes (YYXT, Dayton, DYSYH, Franklin, Yiwu Erleng, Yu6472, TF026, TX9425, Yan89110, Yan90260, Kinu Nijo 6, Naso Nijo, Numar and RGZLL). These genotypes had the lowest average shoot dry weight, were shorter, and had fewer tillers, grains, grain weight and number of spikes and were classified as unresponsive to K^+ fertilization. In comparison to group 2 for example, which increased its average spike number from 1.94 to 2.74 (1.41 fold) between the least and greatest K^+ treatments, group 3 had an average of 1.27 spikes per plant in the lowest K^+ treatment and only 1.61 in the strongest treatment (only 1.27-fold).

In our experiment, the root volume occupied the entire volume of soil in pots and all applied K^+ was efficiently accessed, which is evident from the variation in shoot dry weight. Genotype TX9425 showed a 5-fold change between 0.002 and 20 mM K^+ treatments, but some genotypes like Franklin and Gairdner showed just a 2-fold change between treatments (Table 3.3). Rengel and Damon (2008) studied the genotypic variation in K^+ efficiency, and found in common with the current study that effects of KUE on the vegetative stage of the crop were not necessarily related to grain yield. On the other hand, a constraint of the data is that the reduction in the number of fertile tillers or yield components at maturity might be due to resource limitations, due to the size of the pot that would have restricted the root volume plus may have contributed to variation in soil moisture content. Consequently, additional field experiments would be required to minimise spatial effects on plant growth and to confirm the effect on yield and components of yield (Brennan et al. 2004), although it would on the other hand be difficult to control K^+ in the soil.

K^+ nutrition produces more tiller number in most of the genotypes. Tiller number did not correlate with grain yield (Figure 3.3). The 0.02 mM treatment had the most beneficial effect on tiller number, but some genotypes like RGZLL, Kinu Nijo 6, Yan89110, YU 6472, TF026, YSM1 and YSM3, (which except for the last two are all from group 3 of the PCA), did not respond to increased K^+ availability in the soil. This result indicates K^+ supply above 0.02 mM did not increase tiller number and would be the threshold of deficiency for tiller number for most of the genotypes (Table 3.5). Although it might be expected that enhanced K^+ availability which had improved tiller number would improve enzymatic and photosynthetic activity and shift assimilates to produce more and heavier grains (Ahmad et al. 2009; Akhtar et al. 2003; Hussain et al. 2007; Zeidan & El Kramany 2001), this was only true in some genotypes. Genotypic differences therefore probably explain most of the differences between the current study and those that have found high K^+ application driving grain yield increases. An increase in tiller number that doesn't produce an accompanying increase in grain number per plant or grain weight might in fact be detrimental, as the larger plant size will require more space and therefore reduce the number of plants and yield per hectare. Unproductive tillers will also deplete soil water unnecessarily, thus exposing the crop to greater drought risk. It appears from the results of the current study that in some genotypes of barley high rates of K^+ fertiliser are unhelpful unless the limiting factors that prevent the extra tillers from being productive can also be addressed. It may be that high rates of K^+ that stimulate unproductive tiller growth need to be accompanied by increases in some other nutrients, which will need to be determined

experimentally. The genotypic differences in response to K^+ in the current study demonstrates the need for variety specific recommendations of K^+ application in farmers' fields.

Adaptation of efficient genotypes regarding K^+ efficiency has been a key factor in recommending farmers of different locations and environments. Generally, breeding of crops is conducted in the presence of high K^+ inputs and has neglected to evaluate the genetic variation of genotypes under conditions of low available K^+. Breeding of nutrient efficient genotypes is a novel approach even if they do not always demonstrate phenotypically high nutrient efficient themselves. This is because these genotypes possess the genes that administer the mechanisms associated with nutrient efficiency (Rengel 2005).

The results of the present study revealed there is genetic variation in barley in the adaption to low K^+ availability. The most productive genotypes at low K^+ supply of 0.002 mM was Gebeina, Skiff, YF374 and Flagship and YF374. The less effective genotypes were Dayton, DYSYH, and RGZLL. These genotypes are therefore recommended for mapping DH population, to reveal the QTLs responsible for KUE in barley and for incorporation into barely breeding programs.

Chapter 4 Physiological basis of genotypic variation in KUE in barley (Hordeum vulgare L.)

4.1 Introduction

From the point of view of the role of K^+ in biotic and abiotic stresses, high K^+ is considered as an 'insurance strategy' for improved survival under environmental stress (Kafkafi 1990; Min et al. 2013). Under long-term experiments, plants differ in their K^+ efficiency such that some plants will obtain high yields despite a low soil K^+ supply, whereas others fail (Inostroza-Blancheteau et al. 2017; Meyer & Jungk 1993; Sadan & Claassen 1999; Taha et al. 2000; Zhang et al. 1999). Therefore, screening varieties of barley for their yield in relation to K^+ supply is important for ensuring K^+ is not limiting in crops under stress.

K^+ is the main inorganic cation in the cytosol; the concentration of this ion can be between 100 - 200 mM (Leigh & Wyn Jones 1984). K^+ influences water relations, photosynthesis, transport and enzyme activation. These physiological functions have impacts on crop productivity. Some of the roles of potassium which are important for crop productivity (particularly under abiotic stress) include:

- Plant movements**:** Turgor pressure generated by K^+ drives plant movements such as closing and opening of stomata, leaf movements, and other plant tropisms (Li et al. 2014; Philippar et al. 1999).
- Osmotic adjustment**:** K^+ contributes to a large part of the osmolality of the cell. Inorganic ions are considered cheaper than organic solutes in terms of ATP expenditure (Shabala, S et al 2006). Therefore, K^+ is used as a driving force for rapid cell expansion (Bednarz et al. 1998; Thomas & Thomas 2009).
- Enzyme activation**:** K^+ activates over 60 enzymes which are involved in plant growth. K^+ has the ability to change the physical shape of the enzyme molecule and also helps stabilise pH between 7 and 8 which is optimum for most enzymatic reactions (Anschutz et al. 2014; Leigh & Wyn Jones 1984; Szczerba et al. 2009). Protein metabolism is directly or indirectly influenced by K^+ (Blevins 1985; Lu et al. 2014; Pettigrew 2008).

Taken together, the involvement of K^+ in these physiological processes makes K^+ an important ion in crop growth and development.

It is also important to note that K^+ plays a role in phloem loading with sucrose (Deeken et al. 2002); it has been shown in *Vicia faba* and *Zea mays* that specific K^+ channels are responsible for sugar loading and unloading (Philippar et al. 2003). Potassium concentration in depleted soils can be between 0.1-1mM and can be even lower especially at the root surface (Jungk & Claassen 1986). K^+ deficiency reduces photosynthetic activity, chlorophyll content, and translocation of fixed carbon.

To overcome this issue, K^+ deficiency increases the ability of plants to uptake potassium from soil with low K^+ concentration, due to the increased affinity of the transport mechanism (Drew et al. 1984), as observed in *Arabidopsis* (Shin & Schachtman 2004) and in tomato plants (Nieves-Cordones et al. 2007). Increased sucrose in the leaves of K^+ deficient plants did not cause growth of the roots as the sucrose was not transported to the roots.

There are great genetic differences between and within crop species in both K^+ uptake efficiency (KUpE) and K^+ use efficiency (KUtE.) Plant species differ in their capacity to take up K^+ from the soil (KUpE) and utilize the K^+ physiologically for growth and yield (KUtE) (Fageria et al. 2011; Hafsi et al. 2011; Samal et al. 2010; Trehan 2005; White & Karley 2010). Generally, cereals, legumes and brassicas require less K^+ fertilizer for maximum yield than many vegetable crops (Fageria 2009; Kuchenbuch & Buczko 2011), but cereals and grasses have a greater ability to obtain K^+ and have lower physiological K^+ requirements than legumes plants. The ability to remobilise K^+ from older tissues to younger tissues, critical tissue K^+ concentration and growth rate are all factors which determine the physiological K^+ requirement (Wang et al. 2011). A better ability of plants to acquire and use K^+ would allow them to meet physiological requirements and to produce more biomass under conditions of low K^+ availability (Brennan & Bolland 2004).

Tapping into non-exchangeable K^+ sources is crucial for enhancing K^+ uptake efficiency of crops (Claassen & Steingrobe 1999), and plant species and genotypes within species have shown variations in the use of non-exchangeable K^+ sources (Wang et al. 2011). For example, ryegrass (*Lolium perenne* L.) and sugar beet (*Beta vulgaris* L.) have a higher capacity to mobilize K^+ than wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) do. Variations between crop plants in K^+ uptake are generally not only due to the efficiency of the crop in K^+ absorption, but also in the mobilization of non-exchangeable K^+ by root exudates. Furthermore,

amino acids are also detected in root exudates of wheat and sugar beet and were found to facilitate the release of K^+ from clay minerals (Rengel & Damon 2008). The exhaustion of K^+ in the soil solution around the root zone so that the concentration dropped below a threshold level (10–20 µM) is a crucial signal for the activation of root exudation mechanism (Hosseinpur et al. 2012; Schneider et al. 2013). The organic acids released from the roots are important factors for the weathering of soil minerals by formation of metal-organic complexes and by increasing the exchange of H^+ for K^+ (Hinsinger et al. 1993; Huoyan et al. 2016; Wang et al. 2007; Wang et al. 2011). Understanding the mechanisms governing the release of K^+ from soil minerals is fundamental for developing new approaches for sustainable agriculture.

Efficient genotypes have physiological traits for accessing supplied K^+ (uptake efficiency) or using the supplied K^+ (utilisation efficiency) (Abbadi 2007; Sattelmacher et al. 1994). Potassium uptake in plants is mediated by two types of transport systems, namely a low-affinity K^+ uptake system (LATS) and a high-affinity K^+ uptake system (HATS). Low affinity K^+ uptake is performed by the passive influx of the ion down the electrical gradient through inward rectifying K^+ channels. High affinity K^+ uptake is performed by secondary active membrane transporters by using the energy of H^+ or Na^+ to move K^+ against the electrical gradient (Pettigrew 2008).

For the future, using K^+ efficient plants in combination with optimum K^+ fertilization is a good nutrient management strategy for stable and sustainable farming systems (Rengel & Damon 2008) Eight areas have been put forward as a suggested focus for breeders to improve K^+ efficiency, namely root morphology, root hair formation, root exudates, ability to release K^+ from non-exchangeable pool, kinetics of K^+ uptake, K^+ translocation, K^+ substitution and harvest. The first five traits listed are related to K^+ uptake efficiency and the later three traits are related to K^+ utilization efficiency (Pettigrew 2008).

Plant traits that increase KUtE include: (1) effective K^+ redistribution within the plant, (2) tolerance of low tissue K^+ concentration and (3) maintenance of optimal K^+ concentration in metabolically active cellular compartments under conditions of low K^+ in tissues, (4) replacement of K^+ from its non-specific roles, (5) redistribution of K^+ from senescent to younger tissues, (6) maintenance of water relations, photosynthesis and canopy cover, and (7) a high harvest index. The development of crop genotypes with the above mentioned traits improves both KUpE and KUtE and helps reduce K^+ fertilizer use (Chen & Liao 2017; Oliferuk et al. 2017; White 2013).

The aim of this chapter is to investigate the physiological basis behind genetic variability in KUE in barley reported in the previous chapter.

4.2 Materials and Methods

4.2.1 Genetic material and experimental design

The barley genotypes used are described in Table 3.1. Barley plants were grown under glasshouse conditions and treated as described in Chapter 3, section 3.1.

4.2.2 Non-destructive measurements

In the experiment several non-destructive measurements were taken on the leaf positioned immediately below the flag leaf (also called the penultimate leaf). These measurements were chlorophyll content, chlorophyll fluorescence, and stomatal conductance. These measurements were all taken within a day of each other, all on the same leaf, (the one below the flag leaf), when the plants were at the soft dough/milk stage (Zadok index GS70).

PSII photochemical efficiency (Fv/Fm) is the test most used to measure the effect of stress on maximum quantum efficiency of photosystem II (PSII) photochemistry. Chlorophyll fluorescence was measured using a Chlorophyll Fluorometer (OPTI-SCIENES O1 M704, Hudson, NH 003051, USA). Plants were maintained in darkness for about 60 minutes prior to measurement. The maximum photochemical efficiency of PSII was quantified by measuring the Fv/Fm chlorophyll fluorescence ratio.

The chlorophyll content of the leaf was measured using a Minolta Chlorophyll Meter SPAD-502 (Konica Minolta, Sensing Inc., Sakai Japan). Ten biological replicates were taken. Stomatal conductance was measured using a Decagon Leaf Porometer (Decagon Devices Inc; Pullman, Washington U.S.A.). Plants were exposed to light (400 W) for at least 45 min.

4.2.3 Measurement of K^+ and Na^+ contents in xylem sap and leaf sap using flame photometry

Xylem sap samples were collected at the soft dough/milky stage (GS70). Stems were cut 5–10 mm above the ground and inserted into a Scholander-type pressure chamber (Plant Moisture Systems, Santa Barbara, CA, USA). The cut end was allowed to protrude about 5 mm out of

the chamber through the rubber compression gland. Pressure was applied by filling the chamber with compressed air, thus raising the cell water potential and forcing water into the xylem, and causing xylem solution to exude through the cut ends. This was collected with a micropipette and stored in a 1.5 mL centrifuge tube (Eppendorf). The pressure used to force the xylem sap out of the plant was individually adjusted for each specimen and was typically within the range of 12-15 bars for control samples and 35–40 bars for plants grown under saline conditions. In most cases, between (50-100 µl) of sap was collected from each replicate. To avoid contamination with the phloem sap and the contents of damaged cells, xylem sap was collected within 1-2 min of collecting the plant specimens. The specimens for leaf sap were collected and the sap was extracted as described in the following section (4.2.4).

Leaf sap was diluted 500-fold for flame photometer analysis by taking 0.2 µL of the extracted sap with a 20 µL pipette and adding 10 mL of double distilled water. Xylem sap was similarly diluted, but due to the smaller amount of sample available, only 10 µL was taken, and added to 5 mL of distilled water. Na^+ and K^+ were measured at wavelengths of 589 and 766 nm, respectively, using a flame photometer (JENWAY Bibby Scientific Ltd; Stone Staffordshire; UK).

4.2.4 Measurements of leaf sap osmolality

Leaf sap was extracted from the leaf below the flag leaf on plants at the milky dough stage (GS70). This leaf was excised and stored for two days in a 1.5 ml Eppendorf tube in a freezer at -18ºC. The leaf was then crushed inside the tube using a blunt pointed rod, and the sap that was released was collected immediately with a micropipette, and transferred to a clean Eppendorf tube and re-frozen. The extracted sap was defrosted and centrifuged at 3600 rpm speed for 15 minutes (SiGMA 1-14ED; city; Germany). The osmolality of the centrifuged sap was analysed using a vapour pressure osmometer (Vapro 5520, Wescor, USA), using a 10 µl sample of leaf sap placed into a solute-free paper disc in the sample holder of the osmometer, and commercial standards of 100, 290 and 1,000 mmol/kg. Results were reported in the standard international unit for osmolality, mmol/kg (millimoles of osmolytes per kilogram of water). The vapour pressure osmometer measures dew point depression and does not directly measure concentration of osmolytes in the sap sample, therefore weight or density of sample is irrelevant to the calculation of osmolality by the osmometer.

4.2.5 Estimation of organic solute concentration

The concentration of organic solutes in the sap samples described in the previous sections was estimated by subtracting the contributions of the major inorganic ions (K^+, Na^+ and Cl^-) from the total sap osmolality (Puniran-Hartley et al 2014). The concentration of chloride was not directly measured but was assumed to be 1.3 times the concentration of sodium (Puniran-Hartley et al 2014). Other inorganic ions (including Ca^{2+} and Mg^{2+}) were assumed to be minor and to have no significant effect on comparisons of organic solute concentrations between treatments and genotypes (Wyn Jones et al.1976; Ford & Wilson 1981). This was later validated by measuring Ca^{2+} and Mg^{2+} concentrations in a selection of six barley genotypes under both high and low K^+ treatments (unpublished data).

4.2.6 Statistical analysis

The experiment was a completely randomised block design, with four treatments of potassium and three replicates. The data analysis involved correlation and variance analysis using IBM SPSS Statistics.

Cluster analysis was conducted by XLSTAT software. Hierarchical cluster analysis (HCA) was based on Euclidean distances, a measure of dissimilarity, and Ward's method as a clustering algorithm. The first group contains 11 genotypes including YUQS, ZP2, Flagship, Keel, Schooner, Skiff, Dash, YF374, TX9425, Yan90260 and Naso Nijo. These genotypes showed the lowest leaf Na^+ concentration and osmolality, a medium amount of Gs (stomatal conductance) and xylem Na^+, and highest xylem K^+, leaf K^+, SPAD (chlorophyll concentration) and organic solutes. These are referred to as the low sap osmolality and high leaf K^+ group (Figure 4.1; Figure 4.3).

The second group contains 11 genotypes including YYXT, ZUG403, CM72, Gairdner, Yerong, Yiwu, YSM1, YSM3, Yan89110, Kinu Nijo 6 and RGZLL; these genotypes showed a high Gs and leaf K^+ concentration, a low leaf Na^+ and osmolality, and a moderate amount of xylem K^+, organic solutes and SPAD (chlorophyll content) (Figure 4.1; Figure 4.3).

The third group contains five genotypes including ZUG293, Dayton, Yu6472, TF026 and Numar. These genotypes showed high Gs and leaf Na^+, a low amount of leaf K^+ and osmolality, and moderate leaf Na^+, xylem K^+, organic solutes and SPAD (Figure 4.1; Figure 4.3).

The fourth group contains three genotypes including DYSYH, Franklin and Gebeina. These genotypes had the highest leaf Na^+, osmolality and organic solutes, and the lowest amount of xylem Na^+, xylem K^+, leaf K^+, Gs and SPAD. These genotypes were responsive to K^+ availability in the soil and they showed the highest and lowest amounts of seven of the ten studied traits (Figure 4.1; Figure 4.3).

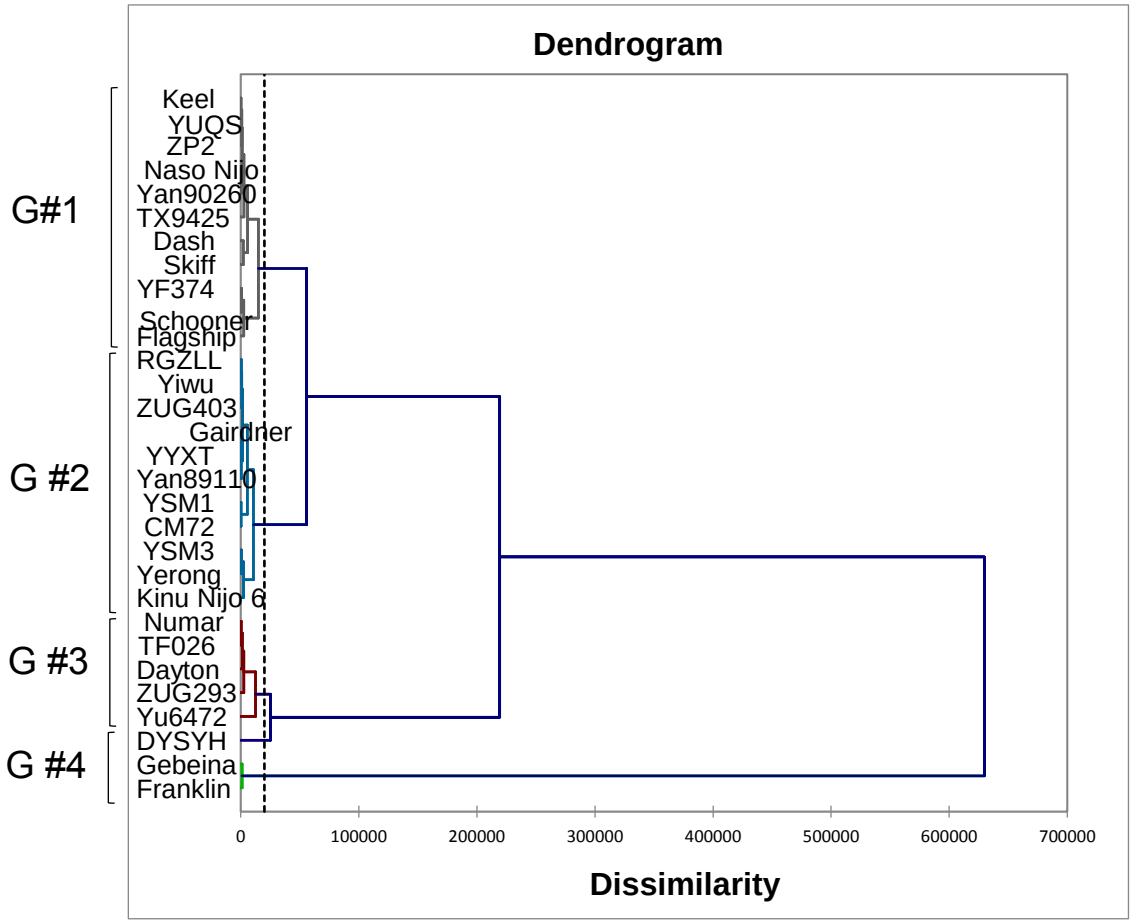

Figure 4.1 : 1 Dendrogram of cluster analysis of 30 genotypes of barley, using Gs (Stomatal conductance), SPAD (chlorophyll content), Fv/Fm (chlorophyll fluorescence), K^+ and Na^+ contents in xylem and in leaves and osmolality, under K^+ supply of 0.002 mM. [The 30 genotypes divide into four groups. Group 1 included 11 genotypes (YUQS, ZP2, Flagship, Keel, Schooner, Skiff, Dash, YF374, TX9425, Yan 90260, and Naso Nijo). Group 2 included 11 genotypeses (YYXT, ZUG403, CM72, Gairdner, Yerong, Yiwu, YSM1, YSM3, Yan89110, Kinu Nijo 6, RGZLL), group3 contend five genotypes (ZUG293, Dayton, Yu6472, TF026, Numar), group 4 included three genotypes (DYSYH, Franklin, Gebeina)]. G# refers to genotype group

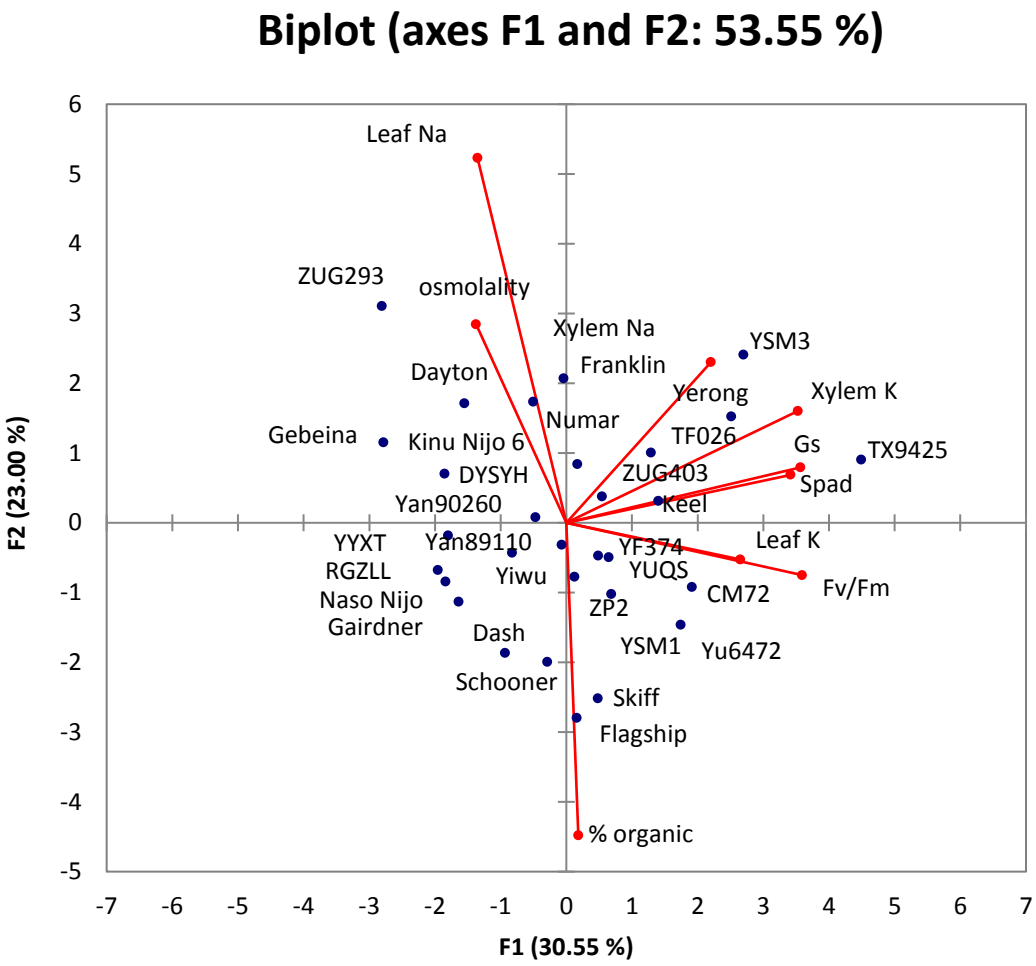

Figure 4.2 Biplot for physiological responses of 30 genotypes of barley based on the lowest soil K^+ treatment (0.002 mM). Names of genotypes are given inside the figure. Physiological measurements shown in the figure are Gs (stomata conductance), SPAD (chlorophyll content), Fv/Fm (chlorophyll fluorescence), leaf Na, leaf K^+, xylem K^+, xylem Na, osmolality, and percentage of osmolality attributed to organic solutes (%organic).

Table 4.1 Eigenvalues from principle component analysis (PCA) for 30 barley genotypes

Factor	Eigenvalue	Variability (%)	Cumulative %
F1	2.75	30.553	30.553
F2	2.07	22.996	53.55
F3	1.326	14.731	68.281
F4	1.144	12.71	80.99
F5	0.741	8.235	89.226
F6	0.469	5.207	94.433
F7	0.261	2.906	97.338
F8	0.237	2.63	99.968
F9	0.003	0.032	100

We used two methods to select the number of PC's;

1- the PC's which explain 70% of variation

2- the PC's which had eigenvalues greater than 1.

PCA analysis found that the first three PC's (principal components) explained approximately 70 % of the total variation. The first, (PC1), explained 30.55% of the variation and had a high positive correlation with xylem K^+, Fv/Fm, SPAD and G_s. Genotypes YUQS, CM72, DYSYH, Gairdner, Yerong, YSM3, Keel, TF026, TX9425, Naso Nijo and RGZLL showed the highest values of PC1 and the highest value of xylem K^+, Fv/Fm, SPAD and G_s. PC2 explained 23% of total variation and was positively correlated to xylem Na^+ and negatively correlated to leaf Na^+ and organic content respectively. Genotypes ZUG293, Dayton, YSM1, Flagship, Schooner, Skiff, Dash and Kinu Nijo6 showed the highest organic solutes and low leaf Na^+. The third PC explained 14.73% of total variation and did not show a high correlation with any of the studied traits.

Osmolality and leaf Na^+ were the most effective traits for classifying genotypes on the basis of physiological traits under low K^+ availability (0.002 mM). All genotypes which were categorised in groups 3 and 4 had higher osmolality and higher leaf Na; these genotypes are K-inefficient genotypes and showed the lowest grain number and grain weight. Genotypes in groups 1 and 2 were seen to be K-efficient genotypes, and had the lower osmolality and higher leaf Na^+ and showed higher grain number and grain weight.

4.3 Results

4.3.1 Leaf K^+ content

An increase in K^+ supply led to an increase in leaf K^+ content in all genotypes, but the size of the response differed according to cultivars (Table 4.2). Under 0.002 mM K^+ application the leaf K^+ content never exceeded 32 mM. By increasing K^+ availability (from 0.002 to 20 mM) a 4- and 39-fold change in the leaf K^+ content was observed in genotypes Schooner and ZUG293 respectively. The greatest difference in leaf K^+ content was observed between 2 and 20 mM treatments. At the lowest K^+ supply (0.002mM) leaf K^+ content ranged from 32 ± 3.9 mM in genotype Yan89110 to 7 ± 1.9 mM in cv ZUG293 (Table 4.2); a 4-fold range in leaf K^+ content. Genotypes Flagship, Keel, Schooner and Naso Nijo showed the smallest changes in response to an increased availability of K^+.

4.3.2 Leaf Na^+ content

In contrast to the leaf K^+ content, the leaf Na^+ content decreased in response to K^+ availability (Table 4.3). Under high K^+ application, the leaf Na^+ content did not exceed 45 mM but under low K^+ application it reached 238 mM. Increasing K^+ availability caused a 2.5 and 28.5-fold change in the leaf Na^+ content in Flagship and Keel respectively. The greatest change of Na^+ content was observed between 2 and 20 mM treatments. With each treatment, barley genotypes showed a broad range of variability in their responses to K^+ supplementation. At the lowest K^+ supply (0.002mM) leaf Na^+ content ranged from 238 ± 10.0 mM in the genotype Franklin to 58 ± 2.7 mM in the genotype Flagship (Table 4.3); a 4-fold range in leaf Na^+ content. After increasing the K^+ supply, a decrease followed in leaf Na^+ content in all genotypes, with the size of the decrease differed significantly between genotypes. The genotypes Flagship, Skiff, Schooner and Dash showed the lowest change in response to increasing K^+ availability.

Table 4.2 Effect of different potassium levels on leaf K^+. Data are mean ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	32±3.8	82±9.8	107±3.2	272±25.1
2	TF026	31±5.9	83±1.1	146±7.3	316±27.8
3	TX9425	31±3.1	75±8.2	138±13.4	261±26.3
4	Yan90260	31±2.4	85±7.8	118±10.3	23026.5
5	Yu6472	29±5.3	77±8.7	154±13.4	325±22.8
6	YSM1	28±2.2	31±4.1	48±7.1	120±16.2
7	YF374	27±2.1	69±8.7	87±11.9	263±3.2
8	YSM3	27±3.0	50±7.4	58±6.4	109±10.9
9	YYXT	27±2.8	67±5.2	81±2.1	324±25.8
10	Yerong	25±3.9	62±4.5	77±7.4	231±21.8
11	CM72	24±2.7	66±4.6	90±1.3	321±21.5
12	Gebeina	24±4.7	52±5.9	64±5.1	292±13.4
13	Schooner	22±2.7	73±7.3	89±4.4	93±18.4
14	Franklin	22±3.9	21±1.6	47±9.3	242±28.7
15	Naso Nijo	21±2.5	69±7.5	122±12.1	96±23.8
16	ZUG403	20±3.1	38±4.7	100±1.6	324±29.1
17	Dash	19±3.3	48±4.8	108±10.1	299±10.6
18	YUQS	18±2.6	37±4.2	48±0.8	115±16.3
19	Skiff	18±3.1	78±9.1	153±10.7	307±22.6
20	Numar	18±1.4	79±6.5	111±13.1	270±1.9
21	Flagship	17±2.2	53±1.8	83±10.7	99±20.7
22	Keel	17±3.3	26±2.2	119±16.7	90±9.9
23	ZP2	17±0.4	38±5.0	49±1.0	311±25.7
24	Gairdner	15±2.0	20±2.2	66±4.3	269±7.2
25	DYSYH	15±0.5	59±11.3	80±0.4	133±23.2
26	RGZLL	12±3.4	63±3.1	78±9.7	234±13.7
27	Kinu Nijo 6	10±1.9	82±9.9	120±11.3	228±23.7
28	Yiwu Erleng	10±2.4	65±6.8	59±9.2	235±46.9
29	Dayton	9±1.3	78±8.9	107±2.6	229±13.8
30	ZUG293	7±1.9	41±6.2	99±1.5	307±19.9

Table 4.3 Effect of different potassium level on leaf Na^+. Data are mean ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	137±6.2	31±2.5	29±5.5	6±0.9
2	TF026	177±19.7	25±4.5	8±2.4	18±1.7
3	TX9425	120±9.5	24±2.2	23±5.5	3±1.8
4	Yan90260	151±11.3	87±12.1	13±0.5	9±1.4
5	Yu6472	92±15.0	75±9.8	41±3.4	12±1.4
6	YSM1	107±1.3	62±5.6	63±6.9	44±2.7
7	YF374	124±6.7	53±6.1	42±5.7	10±2.9
8	YSM3	193±18.6	74±7.4	44±6.1	45±3.2
9	YYXT	136±1.8	75±8.2	89±0.6	14±2.6
10	Yerong	172±19.3	67±6.1	38±7.5	25±2.5
11	CM72	109±9.4	63±6.1	55±0.8	29±2.2
12	Gebeina	220±9.3	60±5.5	52±7.4	43±2.3
13	Schooner	90±16.3	64±7.8	8±2.07	4±1.2
14	Franklin	238±10.0	87±5.5	54±0.6	32±5.5
15	Naso Nijo	132±11.0	56±3.4	42±2.4	9±1.6
16	ZUG403	143±6.70	41±2.8	29±1.6	28±4.4
17	Dash	85±16.5	86±11.5	62±1.5	5±1.7
18	YUQS	121±8.3	72±7.5	91±1.9	33±2.9
19	Skiff	76±11.7	53±5.5	60±2.1	5±2.4
20	Numar	191±13.2	86±11.5	31±4.3	10±2.1
21	Flagship	58±2.7	72±4.6	42±3.5	24±0.7
22	Keel	142±10.9	94±12.6	62±1.9	5±2.3
23	ZP2	120±5.4	62±6.0	105±0.7	20±1.3
24	Gairdner	136±4.1	69±3.2	50±4.4	34±4.6
25	DYSYH	191±14.0	97±2.6	62±1.2	30±3.6
26	RGZLL	119±9.3	96±0.8	40±8.5	37±1.3
27	Kinu Nijo 6	204±1.0	63±4.5	27±2.2	4±2.1
28	Yiwu Erleng	149±10.3	63±7.4	36±6.8	13±2.2
29	Dayton	208±13.9	34±8.5	20±0.3	26±1.1
30	ZUG293	143±6.7	41±2.8	29±1.6	28±4.4

4.3.3 Xylem K^+ content

Xylem K^+ content was affected by K^+ availability in the soil and the extent of the increase in the xylem K^+ content of genotype differed in response to K^+ supplementation. Under low K^+ application the average K^+ concentration in xylem sap never exceeded 5 mM. The availability of K^+ caused a 5 and 20-fold change in the leaf K^+ content in TX9425 and Yiwu Erleng respectively. Most of these varieties showed an increase of around 2-3 times in xylem K^+ content in response to increased K^+ availability. Within each treatment, barley varieties showed a broad range of variability in their responses to K^+ supplementation. Under low K^+ conditions (0.002 mM) xylem K^+ content ranged from 5.03 ± 0.39 mM in cv TX9425 to 1.19±0.11 mM in cv Yiwu Erleng (Table 4.4); the 4-fold difference in xylem K^+ content. The xylem K^+ content increased in all varieties after increasing the K^+ supply. However, the extent of their response differed significantly between genotypes.

4.3.4 Xylem Na^+ content

The xylem Na^+ content decreased gradually in response to K^+ availability. With a high K^+ supply the xylem Na^+ content never exceed 12.4 mM (being highest in genotypes YSMI and Numar), but with low K^+ application it reached 55 mM in (ZUG293). When K^+ availability was increased, YSM1 and ZUG293 showed a 3 and 51-fold change in xylem Na^+ content respectively.

The range of xylem Na^+ concentration under the lowest treatment reached from 55.1±8.6 mM in genotype ZUG293 to 8±2.1 mM in Naso Nijo (Table 4.5); a 7-fold range in xylem Na^+ content. Varieties Skiff, Schooner, Kinu Nijo 6 and Naso Nijo had the lowest xylem Na^+ content under 0.002mM K^+ application and ZUG293, YSM3 and TX9425 had the highest xylem Na^+ content under 0.002mM K^+ application.

Table 4.4 Effect of different potassium levels on xylem K^+. Data are mean ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	3.16±0.13	4.64±0.47	6.67±0.60	28.15±2.18
2	TF026	2.61±0.23	3.48±0.18	5.80±0.51	41.05±2.44
3	TX9425	5.03±0.38	8.64±0.50	13.38±0.78	25.31±3.02
4	Yan90260	3.16±0.13	4.64±0.47	6.67±0.60	28.15±2.18
5	Yu6472	3.32±0.58	5.16±0.39	6.87±0.70	18.38±1.28
6	YSM1	2.45±0.26	3.45±0.26	9.96±0.31	38.18±5.02
7	YF374	3.22±0.24	3.35±0.57	5.06±0.40	30.60±0.93
8	YSM3	4.16±0.28	4.06±0.32	12.16±0.63	21.89±1.49
9	YYXT	2.06±0.11	6.71±0.78	16.28±0.61	30.66±3.76
10	Yerong	2.74±0.19	4.09±0.35	8.03±0.78	25.86±1.70
11	CM72	2.00±0.34	3.55±0.47	5.90±0.45	36.99±2.24
12	Gebeina	2.13±0.14	6.16±0.34	11.77±1.12	26.86±2.30
13	Schooner	2.51±0.28	4.22±0.52	6.77±0.47	12.57±1.23
14	Franklin	2.64±0.17	5.96±0.74	1.80±0.23	26.05±1.74
15	Naso Nijo	2.06±0.42	11.32±0.64	15.8±1.02	31.79±4.47
16	ZUG403	2.93±0.22	13.38±1.00	19.09±0.70	31.08±1.31
17	Dash	1.77±0.06	3.61±0.11	5.09±0.34	22.47±2.37
18	YUQS	2.55±0.15	4.48±0.37	19.67±1.01	21.54±2.03
19	Skiff	2.22±0.52	3.84±0.48	6.83±0.42	11.32±1.01
20	Numar	1.61±0.14	3.67±0.12	5.19±0.37	30.02±2.32
21	Flagship	2.03±0.28	4.09±0.39	10.45±1.07	25.38±1.97
22	Keel	2.90±0.59	4.19±0.37	11.45±0.64	15.19±1.09
23	ZP2	2.06±0.24	3.61±0.50	14.48±0.87	19.51±0.73
24	Gairdner	1.80±0.03	2.61±0.14	4.06±0.28	23.51±1.82
25	DYSYH	1.64±0.12	2.51±0.41	4.03±0.46	20.54±1.37
26	RGZLL	1.44±0.04	3.87±0.20	8.37±0.59	26.03±0.28
27	Kinu Nijo 6	2.97±0.36	4.00±0.51	8.03±0.50	33.70±2.85
28	Yiwu Erleng	1.19±0.11	2.09±0.46	4.26±0.25	23.25±1.10
29	Dayton	1.93±0.20	4.09±0.46	10.93±0.87	18.70±1.36
30	ZUG293	2.22±0.47	7.25±0.57	11.41±1.00	16.93±0.57

Table 4.5 Effect of different potassium levels on xylem Na^+. Data are mean ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	15.3±4.4	10.5±0.6	5.7±0.5	3.7±1.7
2	TF026	37.1±8.3	14.5±0.7	7.0±0.8	2.0±1.8
3	TX9425	60.4±11.7	9.2±0.9	6.2±0.8	3.0±1.0
4	Yan90260	14.4±1.3	4.5±1.0	3.7±3.8	2.7±1.8
5	Yu6472	21.9±1.1	16.3±7.0	7.5±1.7	5.3±1.3
6	YSM1	36.7±6.4	24.6±1.1	21.0±2.1	12.4±1.1
7	YF374	17.1±13.8	11.1±0.8	9.8±2.4	3.4±1.1
8	YSM3	52.9±13.7	35.1±2.0	29.7±4.5	6.7±1.2
9	YYXT	33.2±10.4	15.7±2.5	10.2±2.4	7.0±1.1
10	Yerong	49.7±10.5	13.5±2.4	5.9±1.5	5.7±1.4
11	CM72	35.2±7.3	32.8±5.9	8.8±0.9	8.3±1.4
12	Gebeina	15.6±3.9	15.5±0.9	5.2±1.8	0.7±0.2
13	Schooner	11.5±4.8	8.3±0.6	5.0±2.2	3.5±0.8
14	Franklin	23.1±5.9	15.5±2.4	6.2±0.5	1.1±0.4
15	Naso Nijo	7.9±2.1	4.4±1.2	3.9±0.6	3.4±2.0
16	ZUG403	42.2±2.1	15.9±2.6	10.1±0.2	6.3±1.8
17	Dash	32.8±4.2	5.9±0.8	3.1±1.5	2.7±1.7
18	YUQS	33.2±10.4	15.7±2.5	10.2±2.4	7.0±1.1
19	Skiff	12.5±5.4	11.8±1.2	8.5±2.4	1.1±0.2
20	Numar	17.8±1.7	17.0±1.8	13.9±2.0	12.3±1.7
21	Flagship	26.2±5.9	25.2±1.6	10.0±1.2	2.9±2.0
22	Keel	29.9±10.6	28.3±2.3	17.3±2.2	5.0±0.8
23	ZP2	23.2±3.9	19.0±1.8	7.9±1.73	1.8±0.3
24	Gairdner	27.3±2.8	27.3±2.1	6.6±1.7	6.2±1.6
25	DYSYH	18.0±1.6	14.2±1.2	5.0±0.5	2.5±1.5
26	RGZLL	19.3±0.1	14.1±1.8	10.5±1.8	3.7±1.0
27	Kinu Nijo 6	8.3±3.1	7.9±0.8	6.9±1.1	3.6±1.3
28	Yiwu Erleng	14.4±2.1	7.0±1.1	6.8±1.1	5.8±1.3
29	Dayton	28.9±2.9	19.9±0.6	8.2±1.3	2.4±1.0
30	ZUG293	55.1±8.6	28.6±1.4	16.3±3.7	1.0±0.3

Table 4.6 Effect of different potassium levels on osmolality. Data represents mean osmolality (mmol/kg) ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	705±32.5	692±10.2	748±24.9	916±23.8
2	TF026	864±37.3	734±13.8	775±40.7	880±12.6
3	TX9425	622±44.4	657±40.9	744±44.1	671±10.3
4	Yan90260	640±7.5	649±12.4	654±19.7	735±16.3
5	Yu6472	897±31.4	707±20.3	652±10.4	773±11.3
6	YSM1	754±17.6	631±48.4	708±32.8	901±24.1
7	YF374	590±3.2	629±27.8	667±11.9	715±23.1
8	YSM3	752±23.1	977±75.4	706±65.3	907±23.9
9	YYXT	703±13.1	601±22.3	723±21.5	1157±100.1
10	Yerong	768±13.5	499±12.3	577±11.5	675±10.2
11	CM72	763±22.9	630±7.4	625±12.9	851±34.4
12	Gebeina	1321±262.1	466±17.2	733±56.7	829±18.9
13	Schooner	591±14.2	598±18.9	645±37.4	699±56.0
14	Franklin	1283±40.9	599±17.5	622±13.7	944±50.6
15	Naso Nijo	667±22.3	494±11.7	638±10.6	856±11.9
16	ZUG403	725±0.8	685±60.9	753±22.5	1083±82.1
17	Dash	710±40.1	646±10.5	714±18.1	887±20.0
18	YUQS	671±2.7	469±27.3	575±5.1	824±22.5
19	Skiff	648±10.8	711±106.0	665±46.6	826±72.6
20	Numar	860±8.9	589±20.6	624±35.6	729±21.5
21	Flagship	561±8.2	506±16.4	696±19.4	757±22.2
22	Keel	669±11.9	676±12.1	624±24.6	654±31.1
23	ZP2	636±36.2	622±57.1	733±41.6	919±50.2
24	Gairdner	690±13.5	464±6.0	515±14.4	767±29.1
25	DYSYH	1036±61.0	506±11.6	628±48.9	787±25.6
26	RGZLL	729±30.6	447±14.8	493±13.5	581±16.3
27	Kinu Nijo 6	730±14.0	634±64.0	655±18.6	681±21.6
28	Yiwu Erleng	720±8.1	587±20.3	716±17.8	830±21.1
29	Dayton	829±17.1	635±17.1	769±5.8	712±22.9
30	ZUG293	869±41.5	658±20.7	739±60.8	943±17.6

Table 4.7 Effect of different potassium levels on Fv/Fm (chlorophyll fluorescence). Data are mean ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	0.618±0.056	0.805±0.001	0.801±0.004	0.804±0.003
2	TF026	0.756±0.021	0.802±0.002	0.798±0.002	0.806±0.002
3	TX9425	0.787±0.008	0.809±0.003	0.815±0.002	0.815±0.001
4	Yan90260	0.626±0.044	0.752±0.003	0.727±0.011	0.752±0.010
5	Yu6472	0.734±0.041	0.812±0.002	0.785±0.009	0.806±0.013
6	YSM1	0.696±0.040	0.678±0.020	0.793±0.002	0.778±0.007
7	YF374	0.756±0.021	0.802±0.002	0.798±0.002	0.806±0.002
8	YSM3	0.789±0.003	0.801±0.004	0.769±0.008	0.781±0.009
9	YYXT	0.515±0.048	0.815±0.002	0.814±0.003	0.802±0.003
10	Yerong	0.754±0.027	0.798±0.003	0.787±0.005	0.811±0.002
11	CM72	0.779±0.004	0.810±0.003	0.807±0.003	0.779±0.008
12	Gebeina	0.641±0.054	0.807±0.003	0.799±0.004	0.810±0.003
13	Schooner	0.703±0.069	0.801±0.006	0.799±0.003	0.788±0.004
14	Franklin	0.795±0.020	0.795±0.003	0.781±0.004	0.794±0.004
15	Naso Nijo	0.563±0.052	0.78±0.002	0.775±0.011	0.797±0.003
16	ZUG403	0.416±0.004	0.685±0.048	0.713±0.003	0.823±0.004
17	Dash	0.581±0.059	0.813±0.002	0.809±0.002	0.799±0.002
18	YUQS	0.714±0.020	0.674±0.036	0.796±0.004	0.815±0.004
19	Skiff	0.795±0.007	0.797±0.003	0.823±0.004	0.814±0.006
20	Numar	0.776±0.020	0.769±0.008	0.809±0.002	0.808±0.004
21	Flagship	0.693±0.021	0.814±0.002	0.797±0.003	0.797±0.005
22	Keel	0.766±0.013	0.681±0.030	0.798±0.010	0.792±0.008
23	ZP2	0.411±0.016	0.715±0.028	0.791±0.004	0.790±0.003
24	Gairdner	0.688±0.043	0.816±0.003	0.814±0.001	0.782±0.011
25	DYSYH	0.608±0.044	0.811±0.003	0.772±0.008	0.811±0.004
26	RGZLL	0.592±0.066	0.787±0.013	0.801±0.003	0.798±0.005
27	Kinu Nijo 6	0.638±0.040	0.797±0.001	0.800±0.006	0.760±0.005
28	Yiwu Erleng	0.771±0.012	0.796±0.007	0.806±0.002	0.809±0.001
29	Dayton	0.608±0.044	0.800±0.003	0.801±0.002	0.781±0.006
30	ZUG293	0.384±0.077	0.812±0.003	0.814±0.002	0.800±0.004

Table 4.8 Effect of different potassium levels on chlorophyll content (SPAD values). Data are mean ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	24.6±1.2	39.7±1.37	44.2±2.2	39.6±0.6
2	TF026	32.7±2.9	42.6±1.04	38.7±1.3	46.1±0.6
3	TX9425	41.3±1.1	45.1±1.7	53.8±0.3	52.5±1.0
4	Yan90260	24.0±2.2	32.9±0.9	35.9±0.7	37.1±0.9
5	Yu6472	25.4±2.0	52.2±1.0	45.6±0.9	50.4±0.4
6	YSM1	25.4±1.1	31.4±2.1	40.9±0.8	39.7±0.5
7	YF374	27.1±1.1	37.3±1.1	45.7±1.1	46.3±0.7
8	YSM3	37.6±1.3	37.7±1.1	42.2±0.8	46.1±0.8
9	YYXT	13.2±0.7	43.0±1.	47.4±1.5	46.3±0.4
10	Yerong	38.6±0.9	41.8±0.7	40.7±3.8	49.8±0.7
11	CM72	34.±0.5	43.7±0.8	46.6±0.8	43.8±0.6
12	Gebeina	11.8±0.5	41.3±0.9	42.8±2.0	52.3±1.4
13	Schooner	22.8±1.7	41.2±2.3	49.3±0.7	41.4±1.5
14	Franklin	33.3±4.9	34.8±1.2	41.3±1.1	44.4±1.0
15	Naso Nijo	20.6±2.3	26.7±1.2	34.2±1.0	41.4±0.6
16	ZUG403	20.7±1.3	19.6±0.7	47.5±1.0	46.1±0.6
17	Dash	29.2±2.0	44.1±0.4	44.1±0.5	46.8±0.6
18	YUQS	26.7±0.7	33.5±0.9	48.3±0.9	46.9±1.1
19	Skiff	29.2±2.0	45.5±3.6	41.9±3.8	46.3±1.8
20	Numar	30.8±1.4	35.3±1.3	36.4±0.5	43.9±0.7
21	Flagship	31.9±3.1	43.4±1.3	43.7±0.7	45.9±0.7
22	Keel	33.5±1.2	28.8±1.6	42.1±0.8	42.0±1.1
23	ZP2	24.9±1.1	20.2±1.4	44.3±1.4	48.2±0.8
24	Gairdner	12.1±0.8	48.0±1.7	49.6±0.8	48.9±1.0
25	DYSYH	25.6±2.2	31.5±1.5	34.1±1.1	37.7±0.8
26	RGZLL	27.5±2.3	36.3±1.9	41.3±2.1	45.3±2.1
27	Kinu Nijo 6	29.9±2.2	32.4±1.3	38.6±0.3	41.1±1.6
28	Yiwu Erleng	36.8±1.5	29.6±2.7	38.7±1.5	41.6±1.8
29	Dayton	28.1±1.8	40.7±1.9	42.3±1.1	42.9±1.5
30	ZUG293	20.7±2.0	48.8±1.5	49.4±0.5	53.2±0.7

Table 4.9 Effect of different potassium levels on stomatal conductance (Gs) values. Data are mean ± SE. Genotypes are ordered according to leaf K^+ content.

		Potassium treatment (mM)			
No	Genotype	0.002	0.02	2	20
1	Yan89110	18.3±2.3	34.9±1.6	33.9±2.1	27.4±2.1
2	TF026	31.1±2.5	36.6±1.2	35.7±1.3	46.7±2.3
3	TX9425	39.5±4.9	30.8±2.2	39.5±2.6	47.9±4.5
4	Yan90260	19.5±3.0	28.0±0.7	38.6±3.1	39.0±0.6
5	Yu6472	50.1±1.5	30.5±1.5	24.1±1.1	39.8±0.9
6	YSM1	30.8±1.0	26.6±4.4	41.2±2.4	66.7±1.7
7	YF374	18.9±1.5	28.5±1.1	37.3±1.4	39.4±1.9
8	YSM3	29.6±4.7	20.7±1.2	21.2±1.2	36.0±0.9
9	YYXT	12.7±0.7	26.6±1.8	40.5±2.8	33.8±1.4
10	Yerong	51.8±0.9	44.3±1.7	39.3±0.5	37.7±3.1
11	CM72	52.4±4.2	24.0±0.5	30.7±1.3	45.1±1.8
12	Gebeina	12.4±0.3	31.1±1.8	24.4±1.4	36.9±1.1
13	Schooner	21.2±0.9	29.9±3.1	21.8±1.6	24.8±2.5
14	Franklin	24.4±2.3	32.9±1.3	22.7±2.6	37.3±3.6
15	Naso Nijo	20.6±1.4	26.3±2.5	41.4±0.6	42.8±1.3
16	ZUG403	38.8±1.1	27.6±1.8	23.2±0.6	50.9±2.6
17	Dash	14.1±0.5	51.4±3.2	41.7±3.4	48.2±1.0
18	YUQS	37.6±3.5	17.1±0.4	20.1±2.3	20.0±1.5
19	Skiff	28.7±3.4	18.6±1.1	24.9±1.1	36.5±1.7
20	Numar	45.1±1.3	22.8±0.8	25.9±1.2	29.4±1.1
21	Flagship	20.6±3.4	42.2±1.6	34.5±1.1	37.3±0.6
22	Keel	41.1±1.7	26.1±0.8	27.1±1.8	42.9±1.9
23	ZP2	40.2±0.7	13.6±0.9	31.2±0.9	45.1±2.1
24	Gairdner	13.8±0.5	29.6±2.7	19.4±1.1	25.2±2.1
25	DYSYH	27.4±1.4	16.8±0.7	25.8±2.4	39.5±1.7
26	RGZLL	21.9±1.6	22.4±1.7	37.8±2.4	36.6±1.4
27	Kinu Nijo 6	38.5±2.2	22.6±0.9	27.3±0.6	34.6±2.0
28	Yiwu Erleng	18.2±1.3	32.2±2.2	36.8±3.6	40.2±0.9
29	Dayton	25.2±1.4	21.5±1.0	17.2±1.6	30.2±2.1
30	ZUG293	19.5±0.3	27.8±0.7	16.5±1.6	26.3±0.8

4.3.5 Leaf sap osmolality

If 0.002 mM K^+ was supplied, the varieties DYSYH, Franklin and Gebeina had the greatest leaf sap osmolality (Table 4.6), while the leaf sap osmolality of other varieties was not affected by K^+ treatment. Within each treatment, barley varieties showed a broad range of variability in their responses to K^+ supplementation. The leaf sap osmolality ranged from highest 1321 ± 262 mM/kg in Gebeina to 561 ± 8.2 mM/kg in Flagship (Table 4.6) under low K^+ supply; a 2-fold range in leaf sap osmolality.

4.3.6 Chlorophyll Fluorescence (Fv/Fm)

Chlorophyll fluorescence (Fv/Fm) was not significantly affected by K^+ treatment except in a few genotypes such as ZP2, ZUG293 and ZUG403, which had their lowest Fv/Fm under the 0.002mM K^+ treatment. For each K^+ treatment, barley genotype showed a broad range of variability in their responses to K^+ supplementation, especially under the lowest treatment (0.002 mM). In this treatment chlorophyll fluorescence ranged from 0.795 ± 0.020 in cv. Franklin to 0.384 ± 0.077 in cv. ZUG293 (Table 4.7); a 2-fold difference in chlorophyll fluorescence.

4.3.7 Chlorophyll Content (SPAD)

In general, chlorophyll content was higher under the stronger K^+ treatment, but there were not always statistically significant increases. The largest differences were between the 0.002mM and the 0.02 mM treatment. Differences between 0.02, 2 and 20 mM treatments were comparatively small (Table 4.8), TX9425 was the least affected by K^+ availability, maintaining a chlorophyll concentration of 41.3 ± 1.1 even under 0.002 mM K^+ and thus increasing to only 1.3-fold under 20 mM K^+. The chlorophyll content of TX9425 under 0.002 mm K^+ was 3.5-fold that of the variety with the lowest chlorophyll concentration (Gebeina with only 11.87 ± 0.57).

4.3.8 Cluster analysis

Cluster analysis based on low K^+ (0.002mM) availability resulted in four genotype groups based on physiological traits as described in the following paragraph (Figure 4.1). To understand the effect of traits on grouping we performed an analysis of variance between the four groups. The result showed leaf K^+ and osmolality differed significantly between groups, with the first and forth groups respectively showing the lowest and highest amounts for these traits respectively (the fourth group showing 2-fold that of the first group).

Other traits such as xylem Na^+ and Gs, showed higher differences between groups than leaf K^+ and osmolality did, but the differences were not statistically significant because the variance within groups was high. In general, physiological traits were not able to distinguish genotypes that were efficient in terms of grain production under low K. As a result of the small amount of variance explained by the first two PC's, the PCA biplot was therefore unable to separate the genotypes into the groups that had been made on the basis of physiological traits.

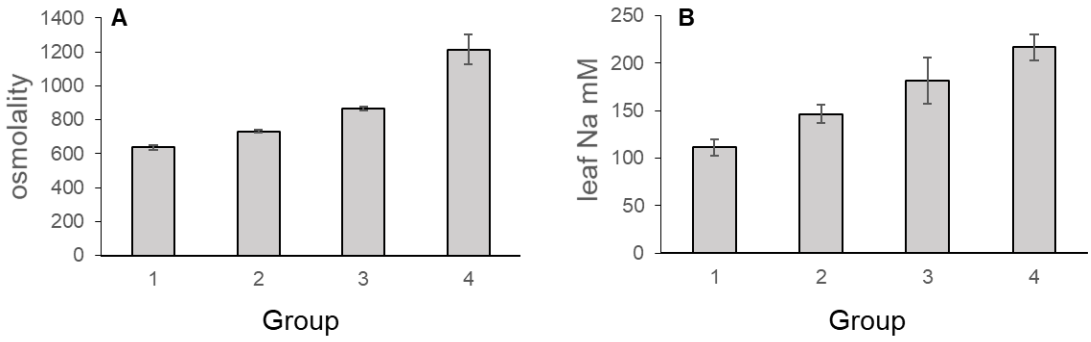

Figure 4.3 Cluster analysis dividing the 30 barley genotypes into four groups according to leaf Na^+ and osmolality under low K^+ availability. Group 1: low leaf Na^+ and low osmolality; group 4: high leaf Na^+ and high osmolality.

4.3.9 Correlation analysis

4.3.9.1 Leaf and xylem K^+

Plants regulate and maintain the cytoplasmic K^+ concentration at approximately 100 mM. Under K^+-deficiency the vacuolar K^+ is depleted to sustain a constant concentration in the cytoplasm, where K^+ can be replaced by Na^+ to maintain K^+ concentration in the cytoplasm. A significant negative correlation between leaf K^+ and grain yield was observed under intermediate concentrations of K^+ availability (0.02 and 2 mM) (Figure 4.4). Grain yield showed positive correlation with the xylem K^+ at all K^+ concentrations except at the highest K^+ supply (20 mM). The positive correlation indicates plants remobilise K^+ to the grains to produce more yield, which is a crucial mechanism for KUE in plants. Under the highest K^+ availability a negative correlation was found between grain yield and xylem K^+, perhaps as a result of reduced uptake of magnesium (Mg) and calcium (Ca) (Figure 4.4).

4.3.9.2 Leaf and xylem Na^+

A correlation between grain yield and Na^+ in leaf and xylem of barley varieties under four different K^+ concentrations showed variable results. A negative correlation was found between grain yield and Na^+ in leaf and xylem at the 0.002 mM application of K^+. The Na^+ in leaf at 0.02 mM K^+ was negatively correlated with grain yield however, a moderate positive correlation at the 0.02 mM K^+ application was observed between grain yield and Na^+ in the xylem. The highest K^+ concentration (2 and 20 mM) revealed a positive correlation between grain yield and Na^+ concentration in leaf and xylem (Figure 4.5).

4.3.9.3 SPAD

The application of different K^+ concentrations (0.002 to 20 mM) showed a significantly positive correlation between grain yield and SPAD values (Fig 4.6ABCD) suggesting the increase in uptake of N during development of barley and promoted grain yield. The highest positive correlation between grain yield and SPAD was observed due to application of 2 mM K^+ concentration (Figure 4.6C).

4.3.9.4 Fv/Fm

The result showed there was a positive correlation between grain yield and Fv/Fm just under the lowest K^+ availability, which indicates that in low K^+ treatment PSII is significantly restricted by K^+ (Figure 4.6E).

4.3.9.5 Stomatal conductance

The significant positive correlation between stomatal conductance and grain yield was found at the lowest K^+ concentration of 0.002 and 0.2 mM (Figure 4.7AB). While, at the highest K^+ concentration of 2 and 20 mM stomatal conductance was not significantly correlated with grains (Figure 4.7CD).

4.3.9.6 Osmolality

A significant negative correlation was observed between osmolality and grain yield at 0.002 and 0.2 mM K^+ concentration (Figure 4.7EF). The application of 2 and 20 mM showed positive correlation between osmolality and grain yield (Figure 4.7GH).

Table 4.10 The correlation matrix between grain weight and physiological parameters (xylem K^+, xylem Na^+, leaf K^+, leaf Na^+, leaf sap osmolality, Fv/Fm, SPAD and Gs. 'ns' denotes not significant, whilst '*', '**', and '***' denotes P-value significance at the 0.05, 0.01 and 0.001 levels.

	Potassium treatments			
Trait	0.002 mM	0.02 mM	2 mM	20 mM
Leaf K^+ (mM)	0.03	0.20 * *	0.12	0.01 **
Xylem K^+ (mM)	0.19 *	0.12	0.15	0.13 *
Leaf Na^+ (mM)	0.18 *	0.03	0.20 *	0.24 **
Xylem Na^+ (mM)	0.01*	0.03 ns	0.20 ns	0.15 ns
SPAD	0.07 ns	0.09 ns	0.21 *	0.13 *
Fv/Fm	0.14 ns	0.00 ns	0.03 ns	0.09 ns
Gs	0.11 ns	0.09 *	0.13 ns	0.00 ns
Osmolality (mmol/ Kg)	0.24 ***	0.00 ns	0.01 ns	0.04 ns

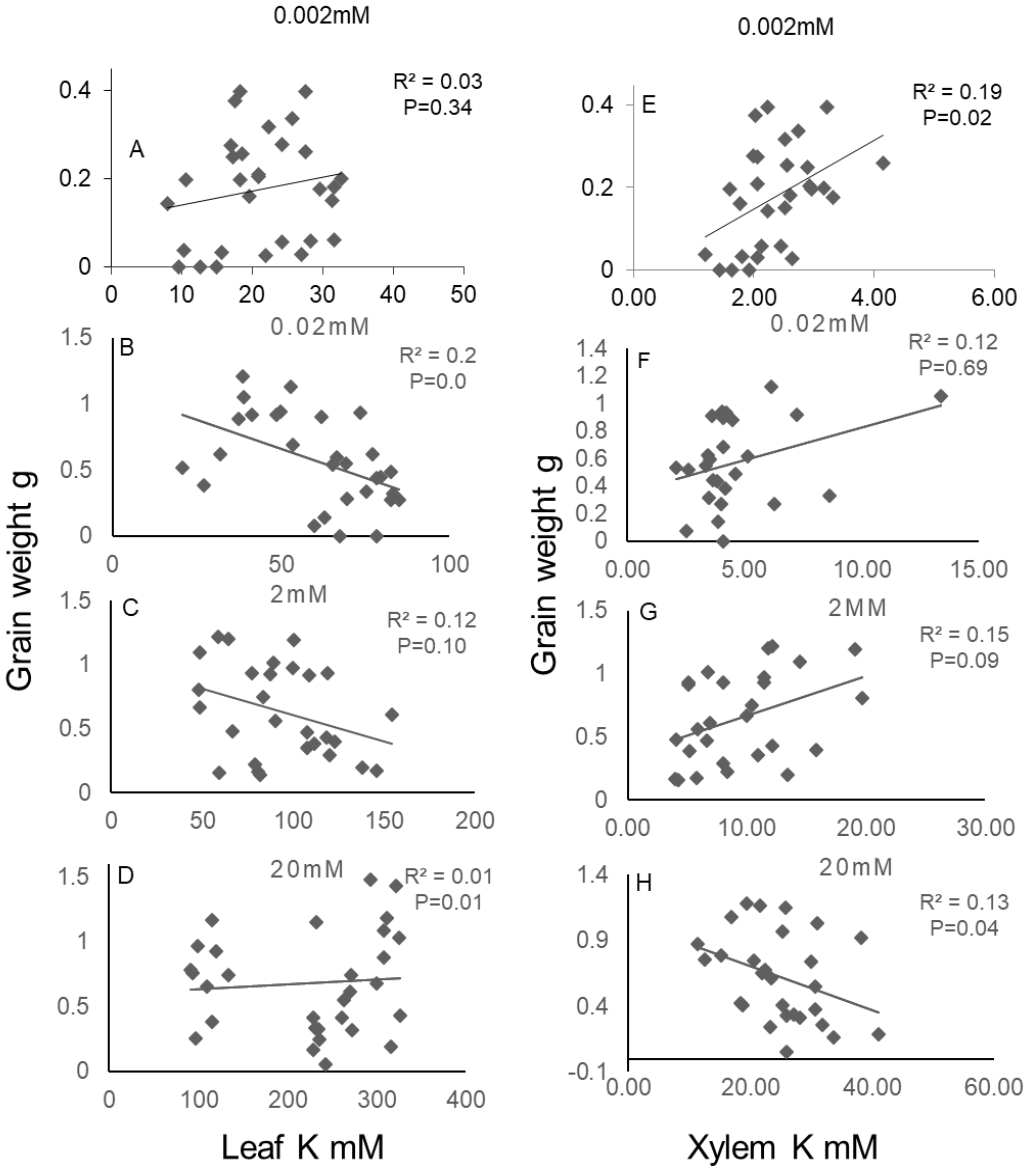

Figure 4.3 Correlation between grain yield, leaf K^+ and xylem K^+ of 30 barley varieties grown under four different levels of K^+ supply. For all K^+ treatments, barley varieties showed aboard range of variability of xylem's K^+ and leaf K^+ in their responses to K^+ supplementation.

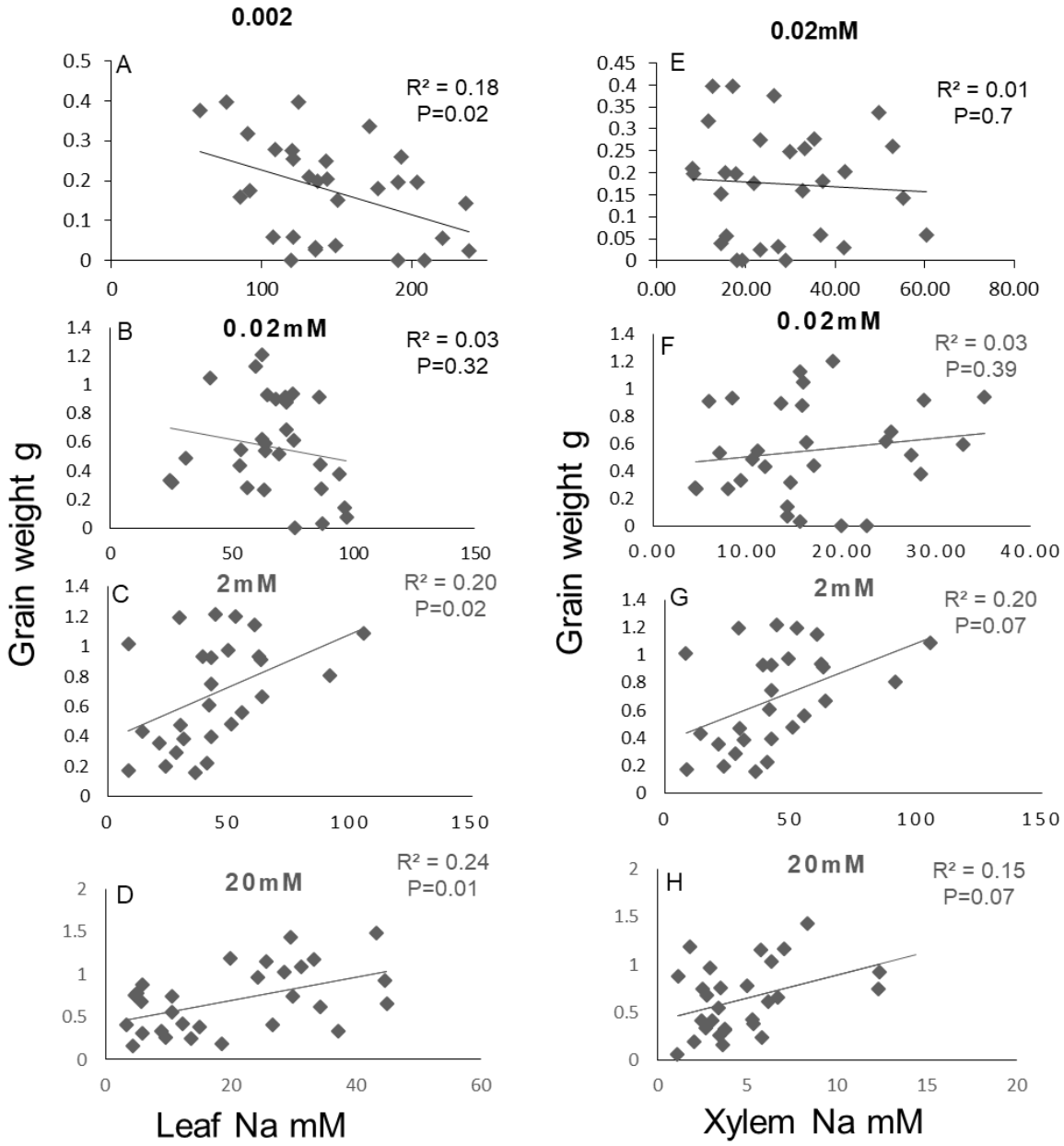

Figure 4.4 Correlation between grain yield and leaf Na^+, xylem Na^+ of 30 barley varieties under four different concentration of K^+. For all K^+ treatments, barley varieties showed aboard rang of variability of leaf's Na^+ and xylem Na^+ in their responses to K^+ supplementation.

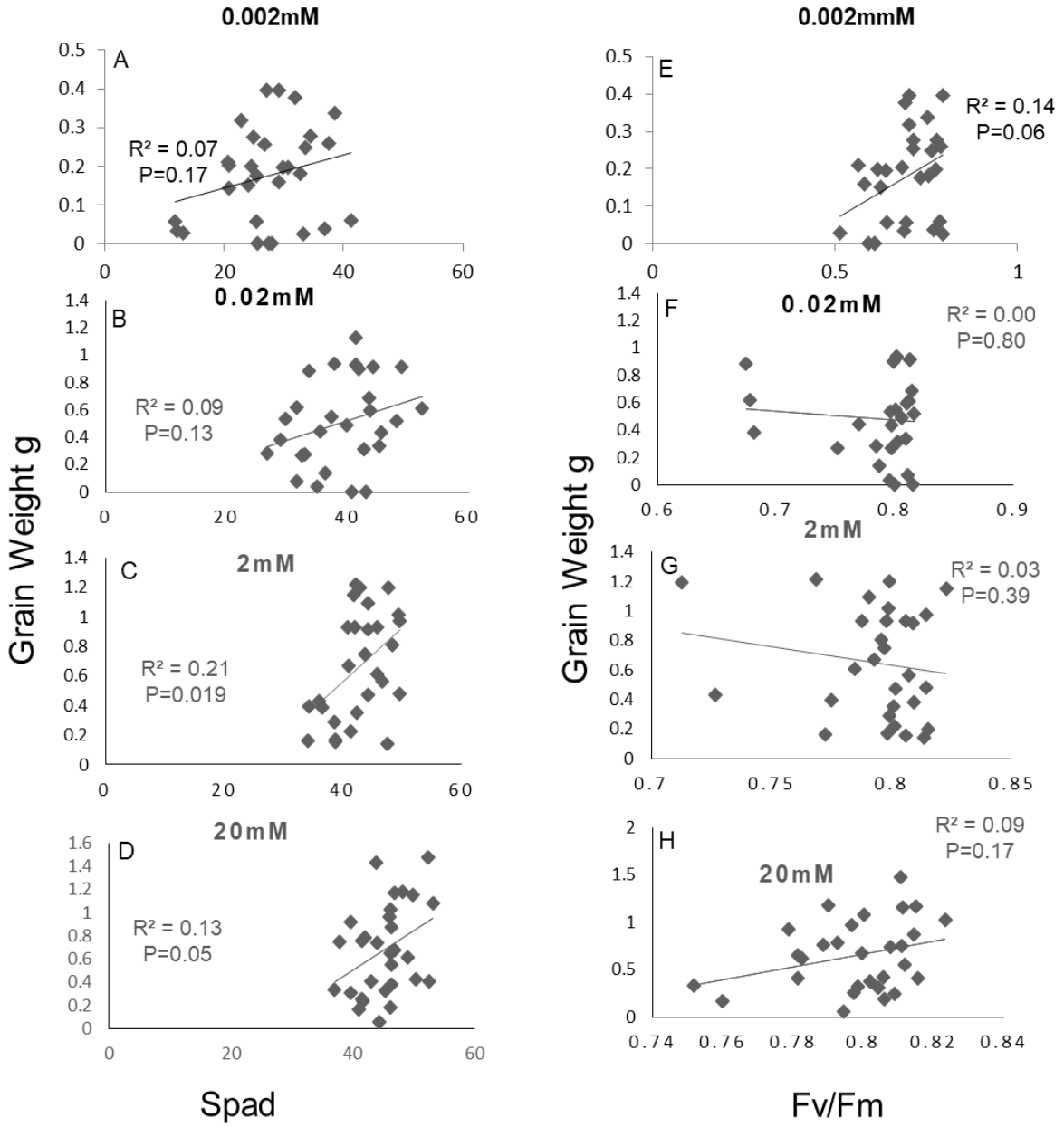

Figure 4.5 Correlation between grain yield and SPAD (chlorophyll content), Fv/Fm (chlorophyll content) of 30 barley varieties under four different concentration of K^+. For all K^+ treatments, barley genotypes showed abroad range of variability of SPAD value and Fm/Fv in their responses to K^+ supplementation.

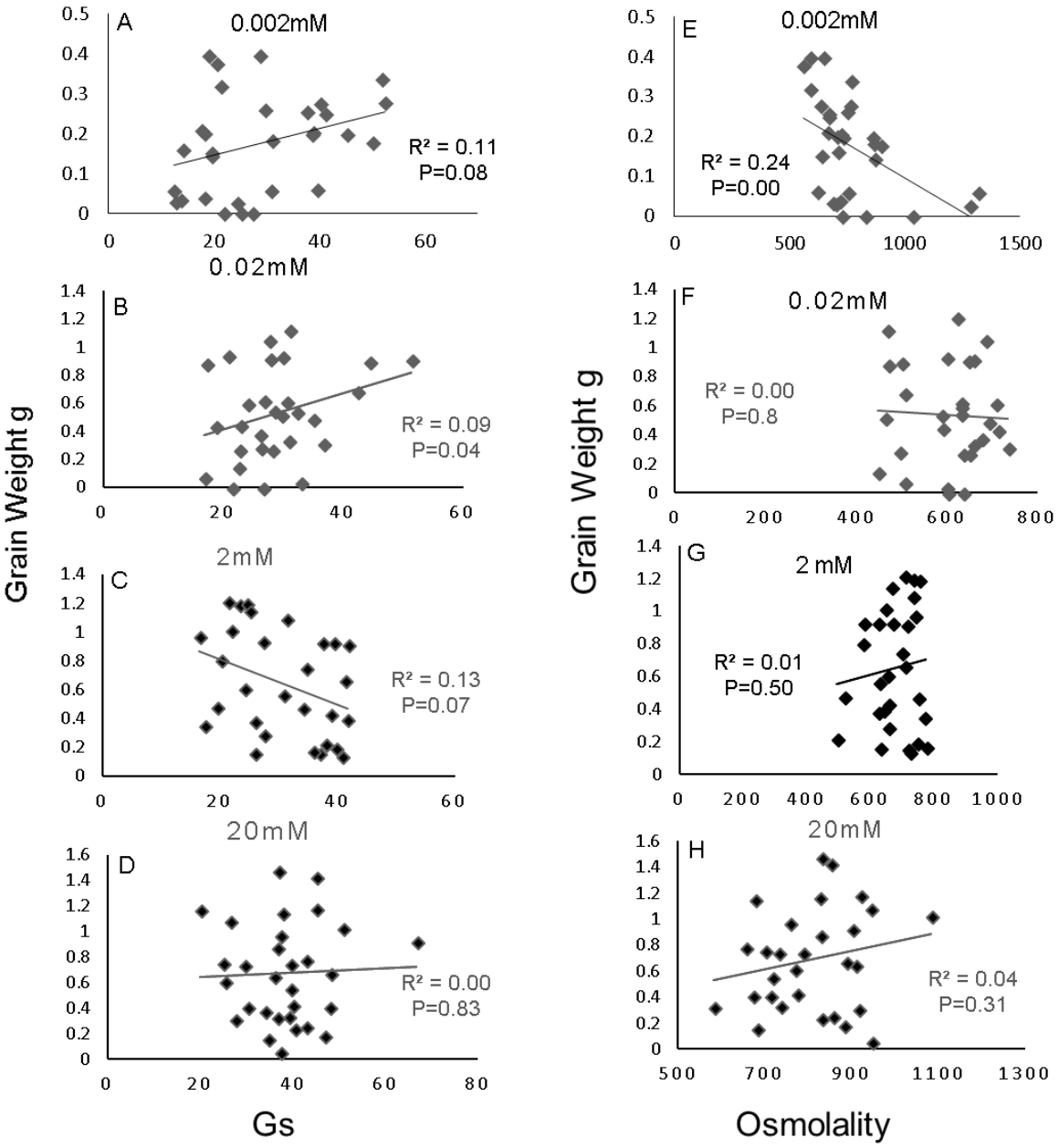

Figure 4.6 Correlation between grain yield and Gs (stomatal conductance), osmolality (mmol/kg) of 30 barley varieties under four different concentration of K^+. For all K^+ treatments, barley varieties showed a broad range of variability of Gs and osmolality in their responses to K^+ supplementation.

4.4 Discussion

Barley response to K^+ supply in the present study varied with K^+ levels. Its uptake in leaves and accumulation in the xylem cells were stimulated, as the application level increased. Leaf sap osmolality, chlorophyll florescence and concentration were increased with K^+ supply. However, Na^+ uptake varied with K-efficient cultivars being more responsive to reduced Na^+ uptake (by low to moderate K^+ supply) along with greater increases in K^+ uptake compared with K^+ inefficient cultivars.

Potassium is the main inorganic cation that plays a major role in functions such as stomata movement, and controls membrane electrical potential, the regulation of cell osmotic pressure and pH homeostasis (Clarkson & Hanson 1980; Oosterhuis et al. 2013). Adequate concentrations of K^+ is needed for optimal protein synthesis, photosynthesis and enzyme activation (Szczerba et al. 2009). Mostly K^+ deficient conditions restricted the optimal plant growth leading to a strong decrease of K^+ content (Drew & Saker 1984; Gierth et al. 2005; Ma et al. 2012; Nieves-Cordones et al. 2007). In the current study, an increase in K^+ availability led to an increased K^+ accumulation in the shoot, as result of higher amounts of K^+ transported by the roots (Table 4.3).

The grain yield showed no direct correlation with leaf K^+ content, at either lowest (0.002 mM) or the highest 20 mM) K^+ treatments (Figure 4.4). However, a significant positive correlation was observed between grain yield and xylem K^+ concentration under severe K^+ deficiency conditions (Figure 4.4). This suggests the ability to control xylem K^+ loading and re-translocate K^+ to developing sinks is more essential for KUE in barley, as compared with the ability of roots to acquire K^+.

The K^+ content was less than 32 mM in the leaf and 5 mM in the xylem with the lowest K^+ application sufficient to promote plant growth whereas with the highest K^+ supplementation (20 mM), the K^+ contents were about the same (ca 45 mM) in the leaf and xylem sap. The K^+ starvation is known to increases the ability of a plants to uptake K^+ from deficient environment by promoting affinity of transport mechanism as observed in *Arabidopsis* (Shin & Schachtman 2004) and in tomato plants (Nieves-Cordones et al. 2007). When plants detect toward K^+ deficiency, the high affinity K^+ system is activated as a response to the short term deficiency (Wang & Wu 2013). In the present study, a significant variation of K^+ efficiency was found among barley genotypes.

Genotypes which showed the greatest K^+ in leaf and xylem at the lowest treatment of 0.002mM included Yan89110 (32± 3.8), TF026 (31 ± 5.9), TX9425 (31 ± 3.1), Yan90260 (31 ± 2.4), Yu6472 (29 ± 5.3), (Table 4.1) and in the xylem Tx9425 (5.03 ± 0.38), YSM3 (4.16 ± 0.28), Yu6472 (3.32±0.58), YF374 (3.22 ± 0.24), Yan89110 (3.16±0.13), (Table 4.1; Table 4.3) which had high uptake of K^+ in barley in leaf and xylem from the highest to the lowest respectively. The genotypes having better ability to accumulate K^+ have highest ability to absorb and use K^+ through adequate supply (Zhao et al. 2006).

The trend in the leaf Na^+ was generally opposite to the trend in the leaf K^+. Genotypes that yielded well under low K^+ (group 1 and 2) showed the lowest leaf Na^+, and the lowest yielding plants (groups 3 and 4) showed high leaf Na^+ (Figure3F). This perhaps indicates plants that are not capable of achieving a suitable concentration of leaf K^+ rely on Na^+ to partly replace K^+ for the opening and closing of stomata, which is important for regulating an internal water balance. However since an ability to replace K^+ with Na^+ would generally be seen as a positive factor in K^+ efficiency, an alternative and more likely explanation is that an important aspect of K^+ efficiency is an ability to exclude Na^+ from leaves so as to maintain an appropriate Na^+ : K^+ ratio. Otherwise, chemical competition between Na^+ and K^+ will exacerbate the K^+ deficiency and result in Na^+ toxicity.

It was found in the first three groups that Na^+ in the xylem increases as the ability of the group to yield under low K^+ decreased. This might be explained as Na^+ retrieval from the xylem being one of the driving forces for K^+ loading to the xylem (Anschutz et al. 2014), or might again be indicative of the importance of Na^+ exclusion and Na^+: K^+ ratio in the ability of barley plants to function under low K^+. However, this trend was broken by group 4, which performed poorly under low K^+ treatment despite having low xylem Na^+. This might be interpreted as a problem with the xylem loading process. An important observation in the current study is that the Na^+ uptake and transport are reduced by higher external K^+ availability. Potassium is able to ameliorate Na^+ ion toxicity (Akram & Akram 2009), and high K^+ : Na^+ is favourable for ionic homeostasis in plant cells under stress favourable for plant growth (Cuin et al. 2003).

In contrast to the K^+ content, the Na^+ uptake in leaf and xylem decreased in response to K^+ availability in the rhizosphere. Potassium in the soil strongly restricts the uptake and transport of Na^+. Under high K^+ application the Na^+ content never exceeded 45 mM in the leaf and 12 mM in

the xylem but under low K^+ application its contents reached 238 mM in leaf and 55 mM in xylem, which demonstrates the antagonistic relationship of K^+ and Na^+. This observation might be associated with an efficient K^+ induced mechanism at root level, where there is a high affinity for K^+ compared with Na^+. This high affinity for K^+ is characterized by a selectivity for K^+ in relation to Na^+ that was prominently higher when K^+ was in greater supply. Similar results have been reported by (Ma et al. 2012).

The results of the current study suggested a competitive interaction between K^+ and Na^+ and their transport into plant parts. Similar results were reported by (Rodrigues et al. 2012) where, under a similar K^+ concentration (10 mM) in the rhizosphere, plants showed a higher selectivity of K^+ over Na^+ and higher rates of loading K^+ into the xylem compared with Na^+. This favourable ionic homeostasis induced by the external K^+ levels was capable of improving photosynthesis and chlorophyll contents and fluorescence. Previous experiments concluded that Na^+ influx in plant root might be facilitated by the high affinity of the K^+ transport system (Dreyer & Blatt 2009). The plant exposed to mild Na^+ availability may depend on K^+ transporters from the HKT family whereas, under high Na^+ availability the Na^+ influx may involve K^+ channels from the AKT/KAT family (Wang et al. 2007).

Under conditions of K^+ deficiency, plants increase expression of high affinity K^+ transporters HKT (Anschutz et al. 2014). Some transporters function as Na^+-K^+ symporters as revealed in the present study where Na^+ encouraged K^+ uptake and K^+ encouraged Na^+ uptake. However, at high external K^+ availability, some of these transporters become K^+ uniporters which are unable to transport Na^+ (Benito et al. 2014).

(Box & Schachtman 2000) reported that with wheat under K^+ deficient conditions, Na^+ present at low concentrations activated the K^+ symporters to increase the K^+ uptake, although it was concluded this was functionally a minor process for K^+ uptake. In the present study, KUE of barley cultivars decided the response to low Na^+, and the effects of Na^+ on transporters that was obscure in the study of Box & Schachtman (2000) was plainly seen in K-efficient barley genotypes. It has similarly been found in wheat that the KUE of a genotype determines whether or not Na^+ can be used to stimulate K^+ uptake under K-deficient soil conditions (Krishnasamy et al. 2014).

The osmolality of barley plants showed an increasing trend towards the lowest performing group under limited K^+ supply conditions (Figure 4.3A; Figure 4.5E-H). In the current experiment, a

broad range of leaf sap osmolality was observed within each K^+ treatment. Under the lowest K^+ supply, a 2-range in leaf sap osmolality was observed, from 1321 ± 262 mM kg^{-1} to 561 ± 8.2 mM kg^{-1}. These results are similar to those of Boscari et al. (2009), who studied K^+ channels during barley leaf growth and development. Leaf sap osmolality drives cell expansion through the continuous uptake of water (Fricke 2002). The K^+ used by cells at concentrations more then 50-100 mM generates osmotic pressure that varies between tissue and cell types (Fricke et al. 1996; Karley et al. 2000; Pottosin et al. 2014). K^+ is the main osmoticum in leaf epidermal cells of grasses contributing 50% of osmotic pressure (Fricke et al. 1994; Wu et al. 2015).

The chlorophyll fluorescence and concentrations in many genotypes were not significantly affected by K^+ supply, even when it was as low as 0.002 mM, but some genotypes did show reductions. The greatest change in chlorophyll fluorescence and concentration between any two adjacent treatments was found between the two lowest treatments, 0.002 and 0.02 mM K^+ (Figure 4.6). The increase in chlorophyll fluorescence and concentration could be because of the involvement of K^+ in enzyme activation, protein synthesis, cell metabolism, and photosynthesis.

Genotypic differences in K^+ use efficiency also influenced K^+ uptake and stress tolerance: K^+ efficient cultivars were more tolerant of low K^+ availability than K^+ inefficient cultivars. The difference between the K^+ efficient and inefficient genotypes involved not only the uptake of K^+ but also its transport and use (Wang et al. 2007). The mechanisms for genotypic variation in K^+ efficiency may be due to the effective uptake from soil and/or efficient utilization of K^+ (Sattelmacher et al. 1994).

In terms of K^+ utilization efficiency, cultivars may differ in their ability to translocate K^+ from roots to other plant portions (Dong et al. 2015; Sattelmacher et al. 1994). Potassium utilization efficiency can be calculated by taking into account both yield and K^+ concentration in the plant (Gourley et al. 1994; Khoshgoftarmanesh et al. 2010). Several studies have associated potassium utilisation efficiency with genotypic differences in K^+ concentrations in shoots for barley (Sharma et al. 2004; Wu et al. 2011), canola (Damon et al. 2007; George et al. 2002; Tian et al. 2008), and sweet potato (George et al. 2002).

In Australia, two thirds of cultivated land is K^+ deficient (Rengel & Damon 2008), and K^+ fertilizers are important inputs, which increase the cost of production for farmers. On the basis of ability to accumulate leaf K^+ under deficient conditions, the present study suggests that cultivars

Yan89110, TF026, Yan90260, Yu6472, Tx9425, YSM3 and YF374 may be useful to breeding programmes to improve K^+ efficiency. These varieties also generally had high xylem K^+. It should be noted though that most of these genotypes were not amongst the highest yielding genotypes in the lowest K^+ treatments, (Chapter 3), so while they may be useful genetic donors for traits related to K^+ accumulation, they do not necessarily combine this ability with traits related to producing grain during when subjected to potassium deficiency. Two of these 7 genotypes, YF374 and YSM3 combined high leaf K^+ with relatively high yield under K^+ deficiency. YF374 had one of the highest grain yields in the lowest (0.002 mM) K^+ treatment, a moderately high yield in the 0.02 mM treatment, and one of the highest yields with sufficient K^+. YSM3 only had a moderately high yield under 0.002 mM treatment, but one of the highest yields in the 0.02 mM treatment and had a high yield with sufficient K^+. The combination of thriftiness (shown by high tissue K^+ under low K^+ treatment) with relatively high yield under K^+ deficiency, and high yield potential in good conditions makes these the genotypes most likely to be profitable in a breeding programme for K^+ efficiency or K^+ thrift.

Chapter 5 Effect of salinity on physiological and agronomical variation in KUE in barley

5.1 Introduction

Soil salinity is a major agricultural and ecological problem threatening more than 800 million hectares or nearly 60% of the total area of land (FAO 2008). Salt stress affects plant growth and development and reduces yield quality and quantity (Ma et al. 2014; Volkov 2015; Yadav et al. 2012). It causes osmotic stress in plants as a result of a decreased soil water potential and also due to accumulation of salts in the plant cell walls (Munns & Tester 2008). Osmotic stress caused by salt has a very similar effect to that of drought stress. Plant adaptation to both these stresses rely heavily on K^+ availability (Shabala & Pottosin 2014).

Inorganic ions (including K^+) are considered to be "cheap osmolytes", unlike organic osmolytes, such as amino acids, methylamines, polyols and sugars (Shabala et al 2006). As an osmolyte, K^+ plays a central role in regulating stomatal aperture and turgor maintenance in all tissues, and in the prevention of water loss. K^+ is the driving force for the influx of water to the guard cell vacuole to effect stomatal opening (Peiter 2011) by which stomatal resistance is decreased, and CO_2 assimilation is enhanced.

The soil osmotic potential and availability of water to plant roots decrease when the concentration of Na^+ and Cl^- ions becomes high in the soil solution. The toxicity of these ions damages cell organelles, and disrupt enzyme structures when they accumulate in the shoot (Maathuis & Amtmann 1999). Salinity stress also causes accumulation of ROS (reactive oxygen species) in both leaf (Tanou & Molassiotis 2009) and root (Xie et al. 2011) tissues. Both these factors disrupt K^+ homeostasis (Maathuis & Amtmann 1999; Shabala & Cuin 2008). About 8% of all genes are estimated to be affected at the transcriptional level of plant structural organisation by salinity (Tester & Davenport 2003), which makes salinity tolerance a complex multigenic trait (Flowers 2004).

Under saline conditions, plant growth is limited by the cytotoxic effect of Na^+ and insufficient water availability. Plants are intolerant of Na^+ concentrations above 20mM in the cytosol

(Amtmann & Sanders 1999; Blumwald & Aharon 2000; Walker et al. 1996). One of the reasons for Na^+ toxicity is the removal of K^+ from K-dependent proteins, thus leading to the inactivation of those proteins (Munns & Tester 2008). Plants have various strategies to manage Na^+ toxicity. Glycophyte plants use the strategy of Na^+ exclusion (i.e. its removal from the plant back to the rhizosphere). However, this strategy causes an increase of Na^+ concentration in root cell walls and the soil, with an energy consuming and futile cycling of Na^+ across the plasma membrane of plant roots (Malagoli et al. 2008). Plants also sequester cytosolic Na^+ into vacuoles of shoots and roots, although with a various degree of efficiency. Other physiological mechanisms contributing to plant salinity tolerance include reducing Na^+ loading into the xylem and its re-translocation from the shoot via phloem (Munns & Tester 2008).

Na^+ can be used to substitute for K^+ in osmotic adjustment in some plants (mainly, in halophytes). This can be done only if plants are capable of keeping most of the Na^+ away from the cytoplasm by sequestering it in vacuoles. It has been suggested that the slow vacuolar (SV) cation channel (TPC1) serves as a 'unidirectional Na^+ valve' as it is blocked by luminal Na^+ (Ivashikina & Hedrich 2005; Peiter 2011). More recently, a plant's ability to reduce the number of open SV channels was shown to be a key attribute for vacuolar Na^+ sequestration in quinoa leaf mesophyll (Bonales-Allatorre et al 2013ab).

Most salt-tolerant crop species, for example sugar beet and barley, have salt inclusion characteristics (Wakeel 2013). In support of this reported mechanism, a barley cultivar overexpressing a Na^+-permeable influx transporter (HvHKT2;1), which enhances Na^+ uptake, showed an increase in growth under moderate salt application, with increased Na^+ accumulation in the shoot (Mian et al. 2011). Therefore, salt inclusion characteristics, if introduced or enhanced in crop plants, may improve growth and reduce the dependence on K^+ for crops grown on salt-affected soils, since the Na^+ can be safely 'locked up' in the vacuole and utilised to replace K^+ for osmotic homeostasis. For both Na^+-includer and Na^+-excluder crops the high K^+ / Na^+ ratio has to be maintained in the cytosol to protect enzymatic function (Shabala 2007).

Over the last decade, cytosolic K^+ homeostasis and an ability of various plant tissues to retain K^+ under stress conditions have evolved as a novel and essential mechanism of salinity stress tolerance in plants (reviewed by Shabala and Pottosin, 2014; Shabala *et al*., 2016a). Reported initially for barley roots (Chen *et al*., 2005, 2007ab), a positive correlation between the overall salinity stress

tolerance and the ability of a root tissue to retain K^+ was later expanded to other plant species such as wheat (Cuin *et al*., 2008, 2009), lucerne (Smethurst *et al*., 2008, Guo *et al*., 2016), pepper (Bojorquez-Quintal *et al*., 2016), cotton (Wang *et al*., 2016a), cucumber (Redwan *et al*., 2016), and Arabidopsis (Sun *et al*., 2015). This trait also explains the inter-specific variability in salinity stress tolerance (poplar - Sun *et al.*, 2009; mangroves - Lu *et al*., 2013; Brassica - Chakraborty *et al*., 2016), and has recently emerged as a novel (and essentially overlooked) mechanism of salinity tissue tolerance in shoots (Wu *et al.*, 2013, 2015). Differential K^+ retention ability also confers differential salinity stress tolerance between halophytes and glycophytes (Percey *et al.*, 2016).

Yousfi et al. (2009) showed *H. vulgare* (cultivated barley, which is regarded as a glycophyte) had higher K^+ concentration in both roots and shoots as compared to *H. maritimum* (a wild barley, which is regarded as a halophyte). A possible explanation for this observation is that wild barley plants may rely much more on the use of Na^+ for osmotic adjustment purposes. In this context, *H. maritimum* required a lesser amount of K^+ to perform under similar conditions and thus possessed higher K^+ use efficiency (KUE). Interestingly, salinity stress also increased KUE in cultivated barley as well, although to a different extent (by 43% and 37%, respectively). This suggests each species, regardless of its origin and habitat, possess some degree of variability in KUE when exposed to saline conditions. How big is this variability amongst cultivated barley genotypes? To answer this question, we have selected six genotypes with contrasting KUE (high efficiency - YF374, Skiff, Yan 89110; low efficiency - Dayton, DYSYH and Franklin) identified in previous chapters and investigated the impact of salinity on plant agronomic characteristics and physiological and ionic attributes of salt grown plants.

5.2 Material and Methods

5.2.1 Effect of saline conditions on barley

A subset of genotypes from Chapter 3 and 4 were used in this experiment, including three genotypes with efficient use of K^+ (YF374, Skiff and Yan 89110) and three genotypes with inefficient use of K^+ (Dayton, DYSYH and Franklin), with six replicates. Amongst the efficient genotypes, Yan 89110 was not particularly high yielding under low K^+ treatment, but it had very high leaf K^+ concentrations. Skiff was the highest yielding variety in the lowest K^+ treatment

despite having only moderate leaf K^+, and reversed this in the second lowest K^+ treatment, where it had only moderately high yield but high leaf K^+. YF374 ranked highly for both yield and leaf K^+ under low K^+ treatment. All three of the genotypes selected as K-inefficient had very low yields in the two lowest K^+ treatments, and had low to moderate leaf K^+.

Growing media and growth condition details were the same as in Chapter 3, section 3.2.1. The only exception was that only two K^+ treatments, low (0.002mM) and high (20mM) were used. Six weeks after sowing, 300 mM of NaCl was added to modified Hoagland solution which was applied to the plants daily for five weeks.

One pot containing six plants was used for measuring stomatal conductance, SPAD and Fv/Fm at the first leaf below the flag leaf. Fresh and dry weight were measured. Two pots with six replicates were watered with 300 mM NaCl in modified Hoagland solution until the grain stage. Plant height, tiller number, grain number, grain weight and shoot biomass were measured.

5.2.2 Elemental analysis

Thirty-seven days after salt treatment, plants of one pot were harvested, thoroughly washed and then blotted to remove the excessive water. Thereafter, shoots were dried at 60°C in an oven for 48 hours, and ground into powder using a mortar and pestle. Samples of leaves and shoot (0.2 g each) were digested with a mix of 5 ml of HNO_3 $^+$ 1 ml of $HClO_4$. The resultant solutions were diluted to 25 ml using 2 % HNO_3 and filtered. The concentrations of Na^+, K^+ and Ca^+ were determined using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent, 7500a) following a standard procedure.

5.2.3 Statistical analysis

The data collected from all measured parameters were analysed by IBM SPSS statistics 20 (IBM, New York, USA). The Univariate General Linear Model with the Duncan test was used to confirm the significance of differences between treatments and genotypes. The data was also subjected to correlation and variance analysis.

5.3 Results

5.3.1 Grain weight and number

Grain weight and grain number were affected by both salinity and K^+ availability, with the salinity impact being much more severe. Grain weight under control (non-saline) conditions ranged from 0.25 g/plant in Franklin (under low K^+) to 2.62 in YF374 (under high K^+), a 10.5-fold range in grain weight. Under salinity stress, the K-inefficient genotypes failed to produce any grain, with the exception of DYSYH which produced 0.02 g/plant with high K^+. The highest average grain weight produced by any variety under salinity was 0.065 in YF374 (with high K^+). However, YF374 was also the variety with the largest decline of grain weight in response to salinity, as it underwent a 212-fold decrease. The K-efficient genotypes had the higher grain weights under salinity, both under low K^+ and high K^+ treatments. Franklin and YF374 showed the largest decreases in their respective groups (K-inefficient and K^+-efficient) for grain weight in response to K^+ deficiency. The decrease was 6.52-fold for Franklin and 3.95-fold for YF374 (Figure 5.1).

K-efficient genotypes were better producers of grain under salinity with both high and low K^+ application in absolute terms (Figure 5.1A), but in relative terms (i.e. relative to non-saline controls) the K-inefficient genotype DYSYH was the best under high K^+ application, while the other two K-inefficient genotypes (Dayton and Franklin) were the worst (Figure 5.1B). Under low K^+ saline conditions all K-efficient genotypes were superior to all K-inefficient genotypes when grown under saline conditions (Figure 5.1B).

The trends in the grain number (Figure 5.2) were generally very similar to those for grain yield weight, but grain number was not as strongly suppressed by salinity as grain weight was (Figure 5.2B).

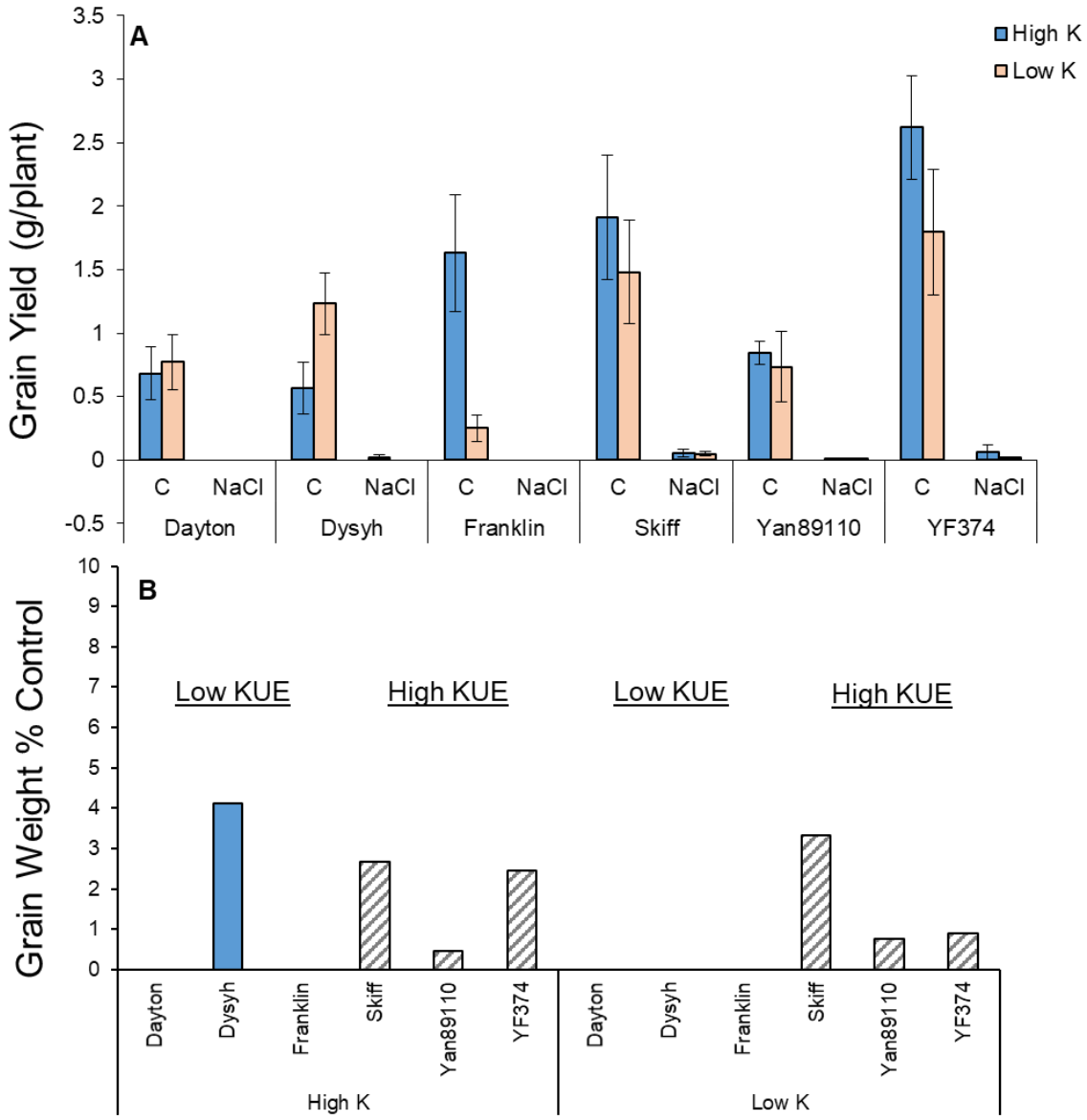

Figure 5.1 (A) Effects of salinity on grain yield in six barley genotypes chosen for being K-inefficient (Dayton, DYSYH, Franklin) or K-efficient (Skiff, Yan89110, YF374). Plants were subjected to salinity stress (300 mM NaCl) or no salinity (Control) under conditions of either high (20 mM) or low (0.002 mM) soil K^+ application. (B) Relative grain yield of barley genotypes grown under salinity (as described for Figure 5.1A), showing grain yield as a percentage of the yield under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

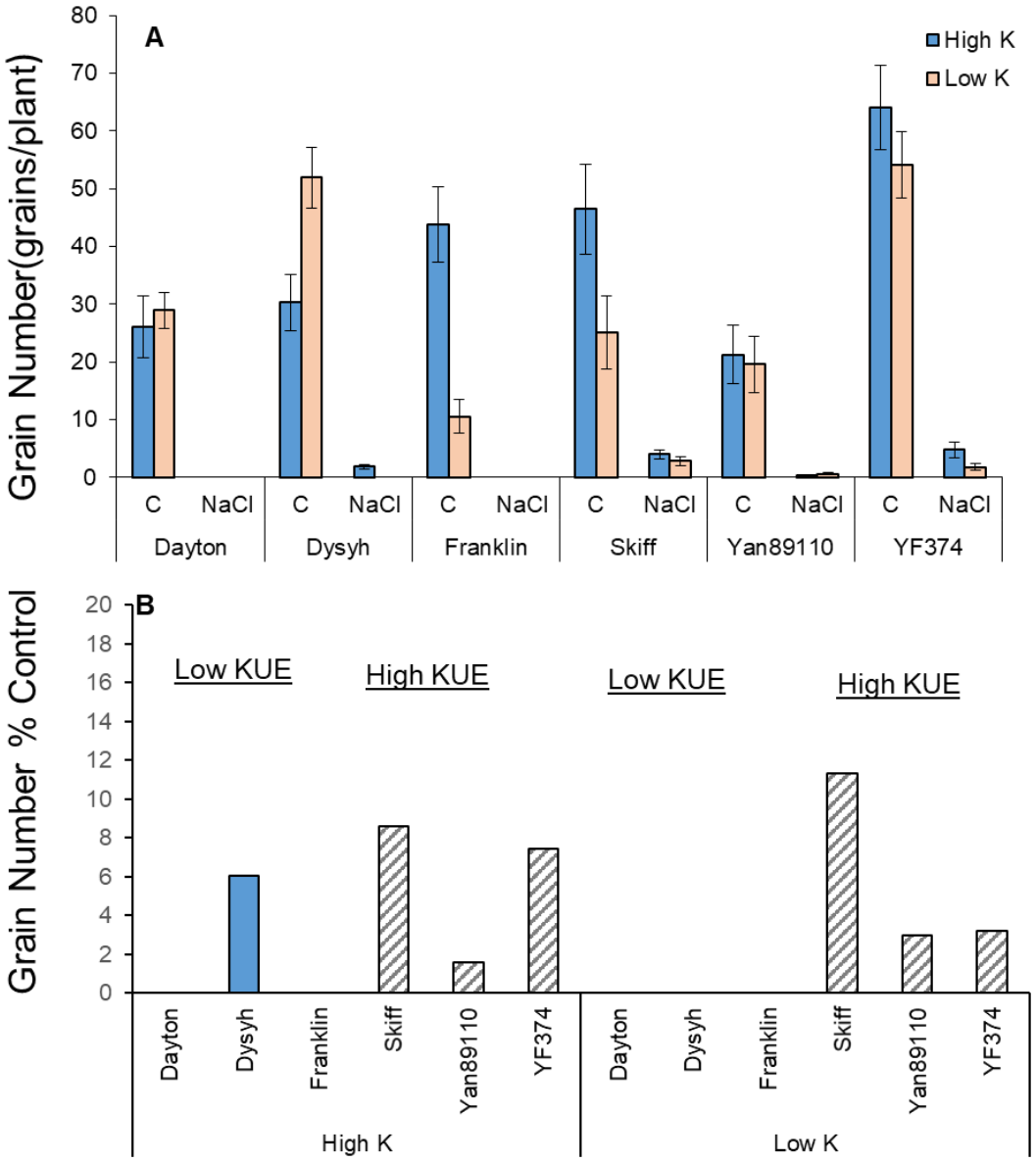

Figure 5.2 (A) Effects of salinity on grain number in six genotypes of barley chosen for being K-inefficient (Dayton, DYSYH, Franklin) or K-efficient (Skiff, Yan89110, YF374). Plants were subjected to salinity stress (300 mM NaCl) or no salinity (Control) under conditions of either high (20 mM) or low (0.002 mM) soil K^+ application. (B) Relative grain number of barley genotypes grown under salinity (as described for Figure 5.2A), showing grain number as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.3.2 Total shoot biomass

Plants grown under salinity stress showed a significant (by over 80%) decrease in the biomass production (Figure 5.3). Under high K^+ supply, the impact of salinity was statistically not significantly different between low- and high-KUE groups (Figure 5.3B). However, under conditions of low K^+ supply, high KUE genotypes outperformed low KUE genotypes by about 2-fold, when grown under saline conditions (Figure 5.3B).

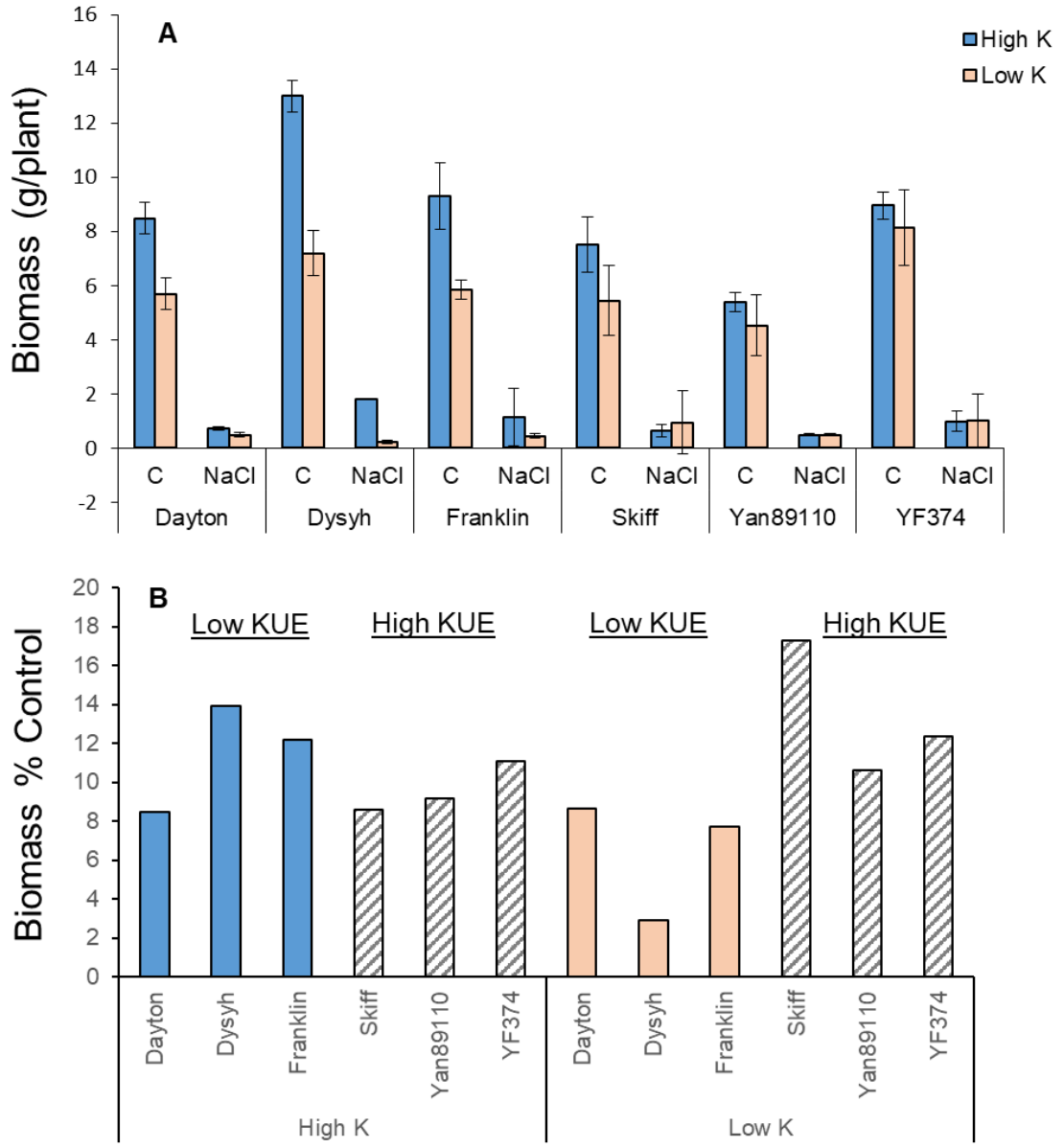

Figure 5.3 (A) Effects of salinity on the total shoot biomass in six genotypes of barley chosen for being K-inefficient (Dayton, DYSYH, Franklin) or K-efficient (Skiff, Yan89110, YF374). Plants were subjected to salinity stress (300 mM NaCl) or no salinity (Control) under conditions of either high (20 mM) or low (0.002 mM) soil K^+ application. (B) Relative biomass of barley genotypes grown under salinity (as described for Figure 5.3A), biomass as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.3.3 Plant height

The impact of salinity treatment on plant height was always greater than the effect of K^+ availability (Figure 5.4A). Plant height was less affected than other traits were by salinity and K^+ deficiency; in fact, in several cases, including both K-efficient and K-inefficient genotypes, and both saline and non-saline treatments, plant height was not at all statistically affected by K^+ treatment. The K-inefficient genotypes had the highest plant heights in the absence of salinity, regardless of the K^+ treatment. Only under the combination of salinity and low K^+ did the K-efficient genotypes appear clearly taller than the K-inefficient genotypes (Figure 5.4B).

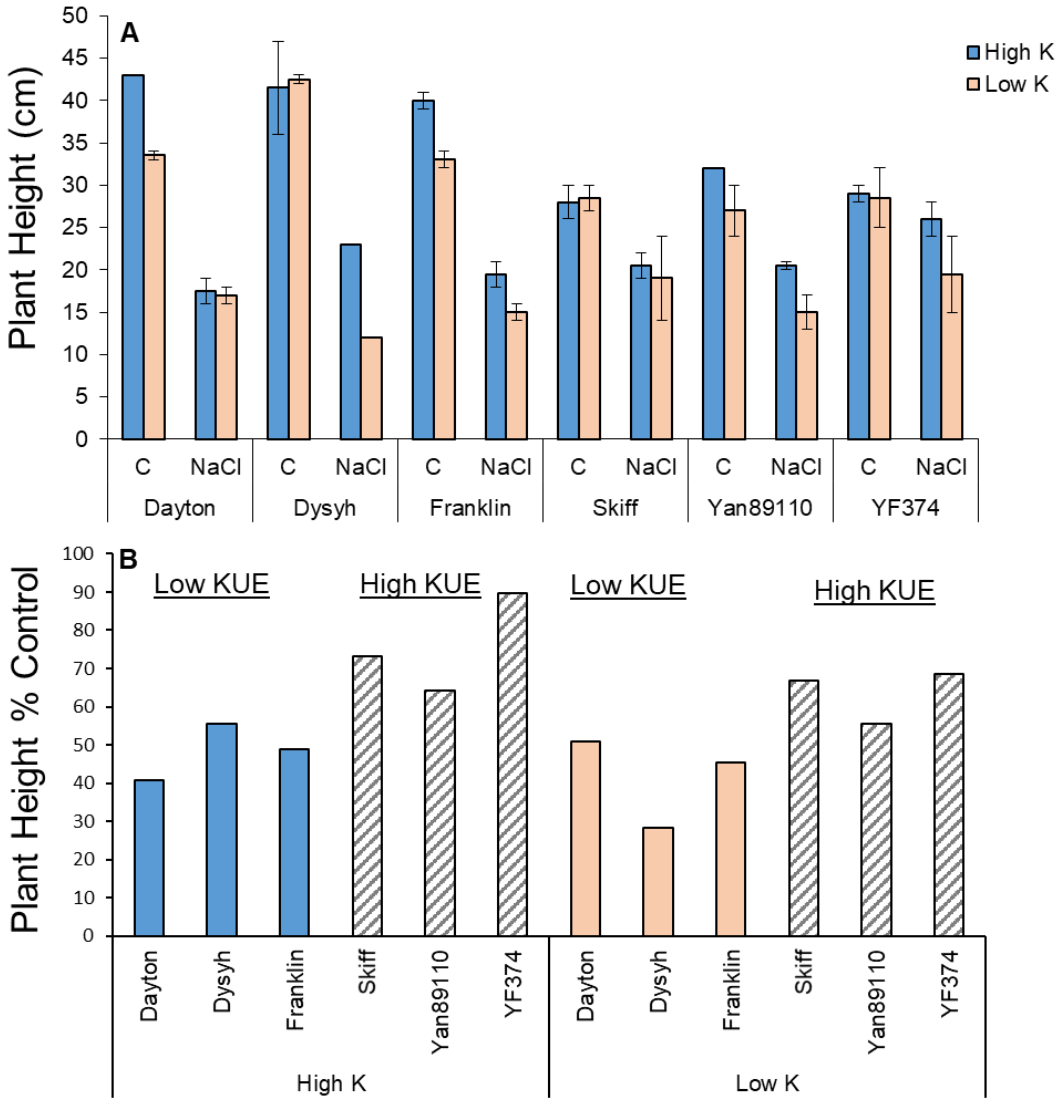

Figure 5.4 (A) Effects of salinity and K^+ treatment on heights of K-efficient (Skiff, Yan 89110 and YF374) and K-inefficient (Dayton, DYSYH and Franklin) genotypes of barley. Seedlings were subjected to one of two treatments of K^+ (low: 0.002 mM; high: 20 mM); in either absence (Control) or presence of salinity stress imposed by irrigating with 300mM NaCl. Plant heights were measured at the time of grain harvest. (B) Relative height of barley genotypes grown under salinity (as described for Figure 5.6A), showing height as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.3.4 Tiller number

The effect of salinity on tiller production was more pronounced than the effect of K^+ availability (Figure 5.5). In fact, where tiller numbers were always reduced by two thirds or more as a result of salinity, a low K^+ treatment appeared to actually stimulate tiller production under both saline and control conditions in genotypes Yan89110 and YF374, and under saline conditions in genotypes Skiff and Franklin, (although not with statistical significance). The last-mentioned genotype is K-inefficient, and the others are K-efficient.

The greatest tiller production under low K^+, both with and without salinity stress, was in genotypes Yan89110 and YF374. However, the higher tiller production from these two K-efficient genotypes did not result in higher fresh weight or dry weight.

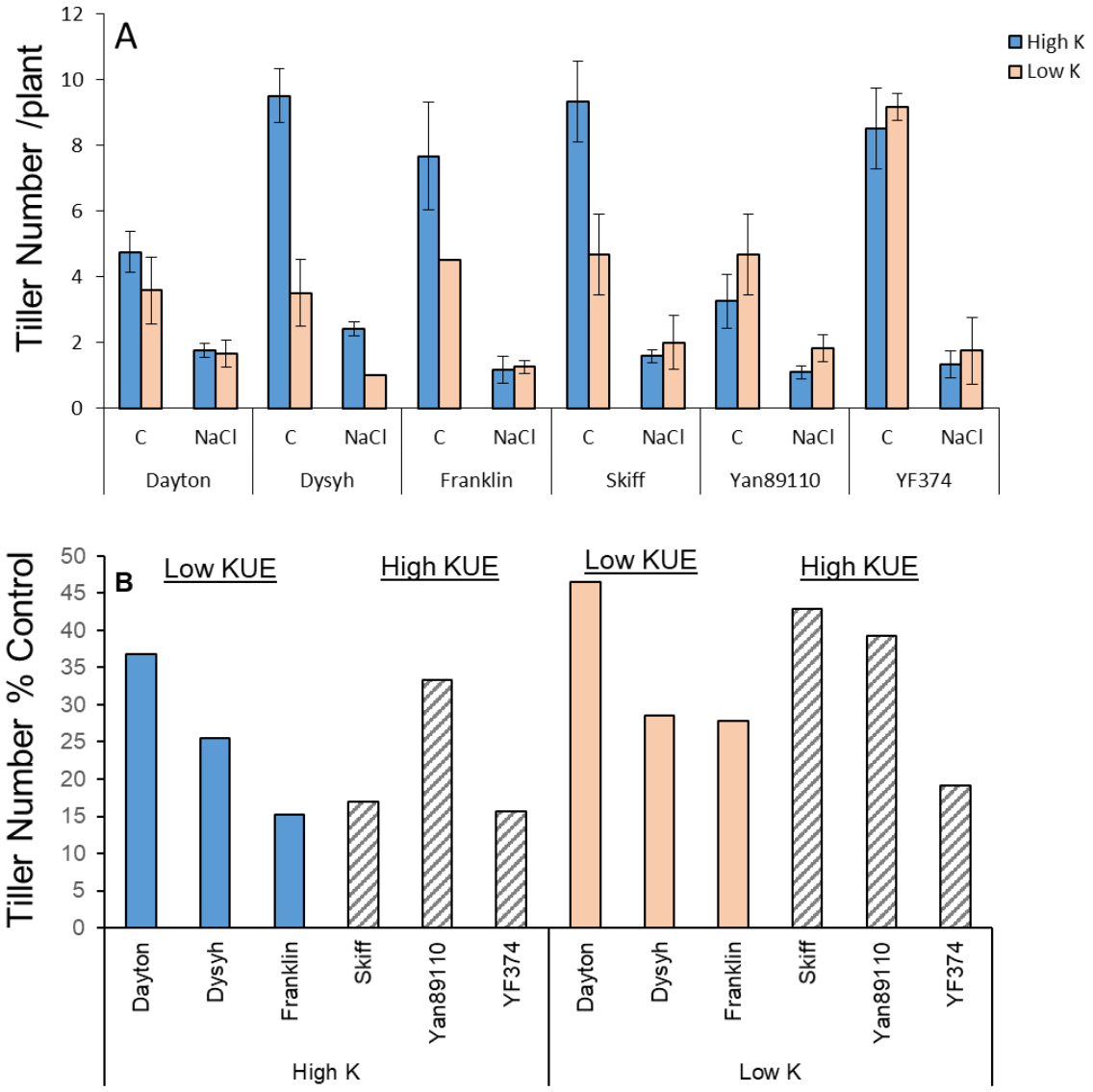

Figure 5.5 (A) Effects of salinity and K^+ treatment on tiller numbers of K-efficient (Skiff, Yan 89110 and YF374) and K-inefficient (Dayton, DYSYH and Franklin) genotypes of barley. Seedlings were subjected to one of two treatments of K^+ (low: 0.002 mM; high: 20 mM); in either absence (Control) or presence of salinity stress imposed by irrigating with 300mM NaCl. Tillers were counted after 5 weeks of the treatment period had elapsed. (B) Relative tiller number of barley genotypes grown under salinity (as described for Figure 5.7A), showing tiller number as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.3.5 Spike number

Salinity has severely reduced the spike number; this reduction was much more pronounced in genotypes with low KUE. When low KUE were grown under conditions of K^+ deficiency, all three low KUE genotypes (Dayton; DYSYH; Franklin) failed to produce any spikes (Figure 5.6b) while in the high KUE group two out of three genotypes produced 30% of the spike number, relative to the control. Interestingly, within the high KUE group, increased K^+ supply led to a decrease in the total spike number (by about 2-fold; Figure 5.6).

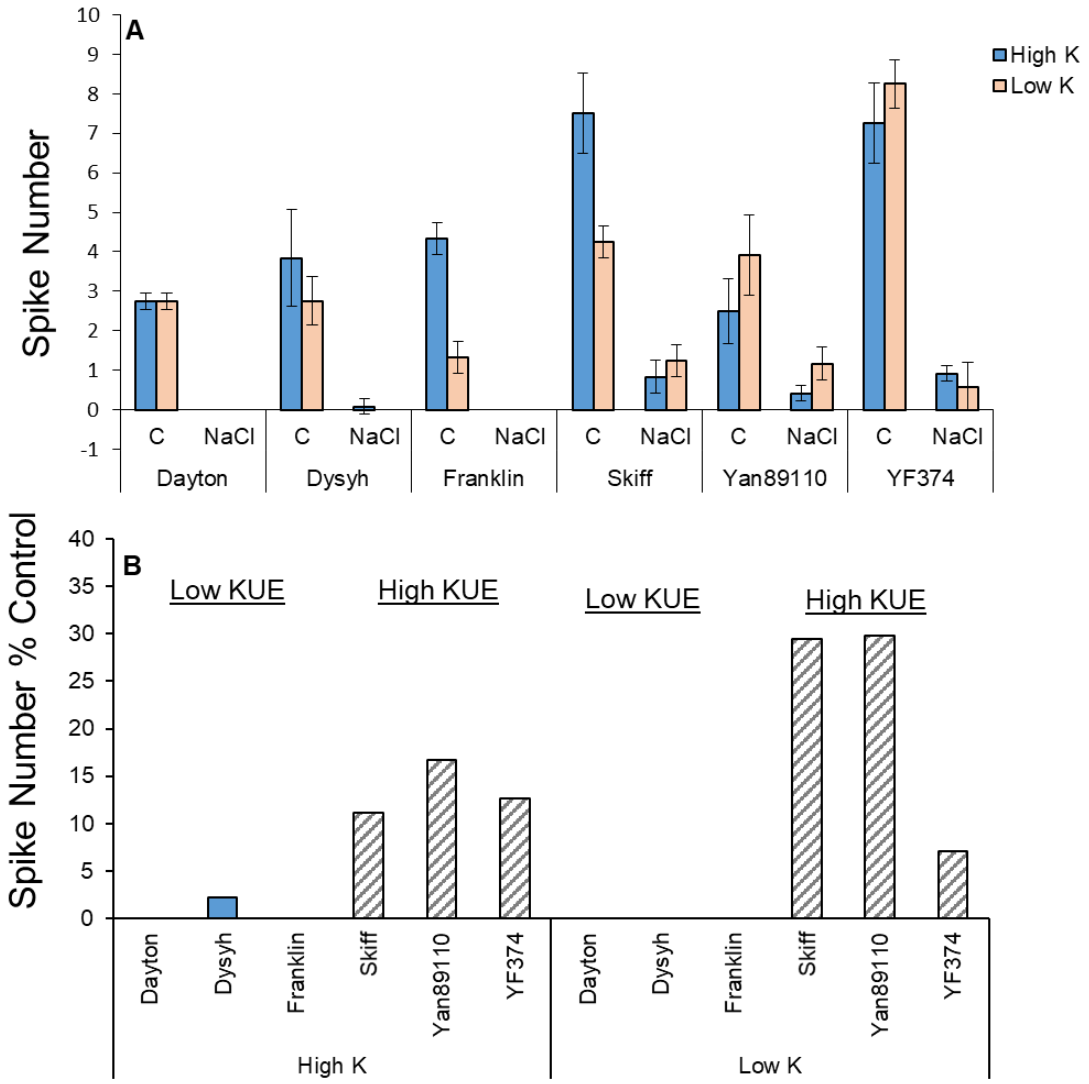

Figure 5.6 (A) Effects of salinity and K^+ treatment on spike numbers of K-efficient (Skiff, Yan 89110 and YF374) and K-inefficient (Dayton, DYSYH and Franklin) genotypes of barley. Seedlings were subjected to one of two treatments of K^+ (low: 0.002 mM; high: 20 mM); in either absence (Control) or presence of salinity stress imposed by irrigating with 300mM NaCl. Spikes were counted after 5 weeks of the treatment period had elapsed. (B) Relative spike number of barley genotypes grown under salinity (as described for Figure 5.8A), showing spike number as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.3.6 Stomatal conductance

Salinity stress has affected leaf gas exchange characteristics, reducing stomatal conductance by about 80% (Figure 5.7). However, no statistically significant (at $P < 0.05$) difference was found between low- and high- KUE groups (Figure 5.7B) except for genotype DYSYH which was an outlier and should be discarded from analysis.

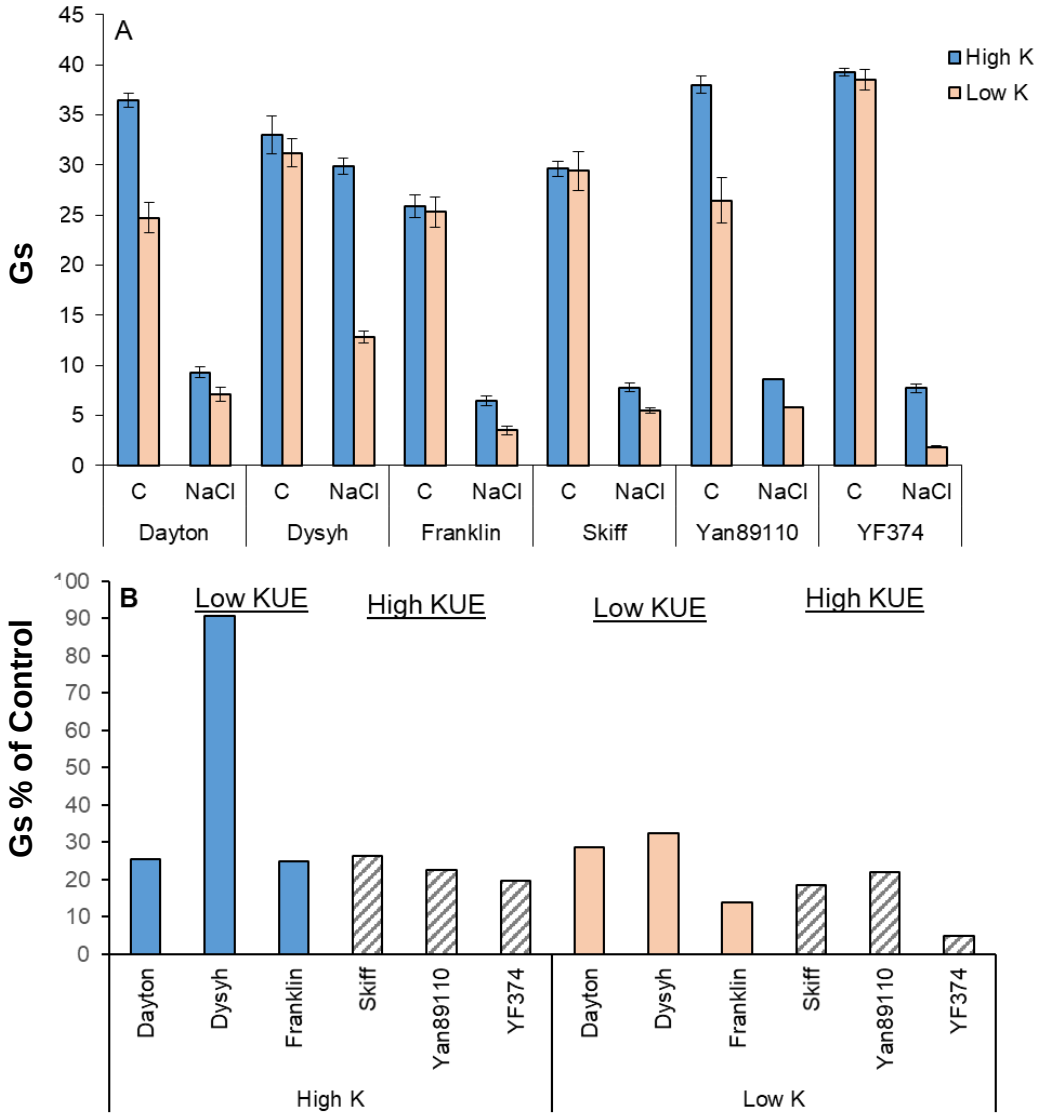

Figure 5.7 (A) Effects of salinity and K^+ treatment on Gs of K-efficient (Skiff, Yan 89110 and YF374) and K-inefficient (Dayton, DYSYH and Franklin) genotypes of barley. Seedlings were subjected to one of two treatments of K^+ (low: 0.002 mM; high: 20 mM); in either absence (Control) or presence of salinity stress imposed by irrigating with 300mM NaCl. Gs was measured after five weeks of the treatment period had elapsed. (B) Relative Gs of barley genotypes grown under salinity (as described for Figure 5.9A), showing Gs as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.3.7 Leaf chlorophyll content (SPAD value)

In the genotypes Skiff and Yan89110 salinity had the highest effect on SPAD, while in the genotypes Dayton, DYSYH and Franklin K^+ availability had the greatest effect on SPAD. Skiff had the highest SPAD in the control treatment and showed the lowest SPAD under salinity stress (about 3-fold change) and was thus the most responsive genotype to salinity stress. The highest and lowest relative changes between salinity and control were observed for Skiff (5.25 times) in high K^+ and Dayton (1.02 times) in low K^+. DYSYH showed the highest difference between high and low K^+ availability (2.26-fold change) under salinity stress (Figure 5.8).

Whereas K-efficient genotypes were affected more by salinity than by K^+ deficiency, and the reverse was true for K-inefficient types. The effect of salinity on chlorophyll content was not significantly different between K-efficient and K-inefficient genotypes (Figure 5.8B).

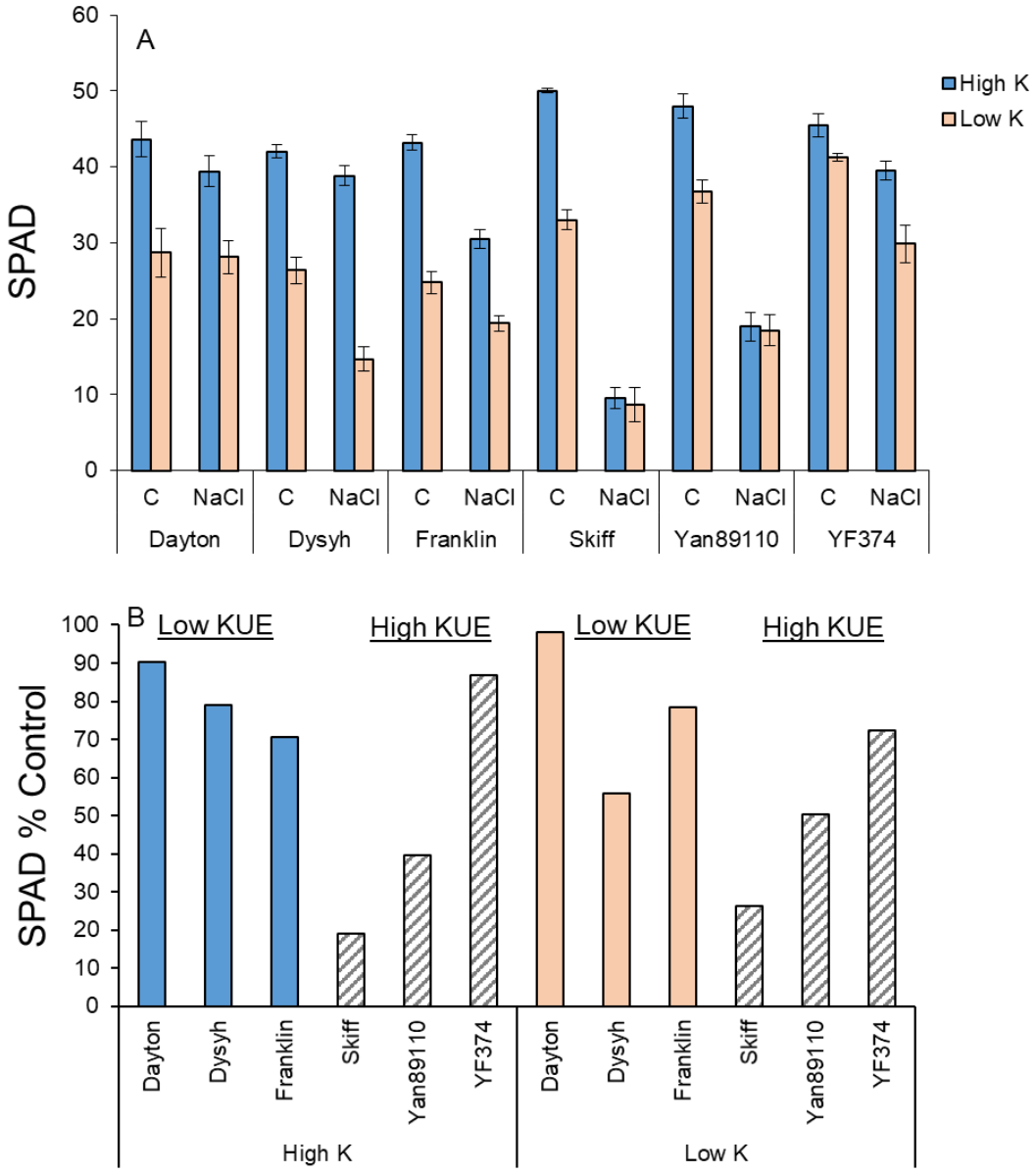

Figure 5.8 (A) Effects of salinity and K^+ treatment on chlorophyll concentration (SPAD value) of K-efficient (Skiff, Yan 89110 and YF374) and K-inefficient (Dayton, DYSYH and Franklin) genotypes of barley. Seedlings were subjected to one of two treatments of K^+ (low: 0.002 mM; high: 20 mM); in either absence (Control) or presence of salinity stress imposed by irrigating with 300mM NaCl. SPAD was measured after five weeks of the treatment period had elapsed. (B) Relative SPAD of barley genotypes grown under salinity (as described for Figure 5.10A), showing SPAD as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.3.8 Shoot ion content

Salinity stress has very little impact on shoot sap K^+ concentration in plants grown under luxury K^+ supply (Figure 5.9), with all genotypes showing some modest (10-20%) increase in sap K^+ concentration (Figure 5.9B). Under low K^+ availability conditions plant responses to salinity showed much more genetic variability. No clear trends were observed, although on average sap K^+ content was higher in K^+ the efficient group (Figure 5.9B).

A much more striking difference was reported for the shoot sap Na^+ as a function of K^+ availability (Figure 5.10). When plants were grown under luxury K^+ supply, sap Na^+ concentration increased by about 10-fold (Figure 5.10B). This increase was however only 2-3-fold in plant grown under low K^+ supply. No significant differences between the two groups (e.g. K-efficient and K-inefficient genotypes) were observed

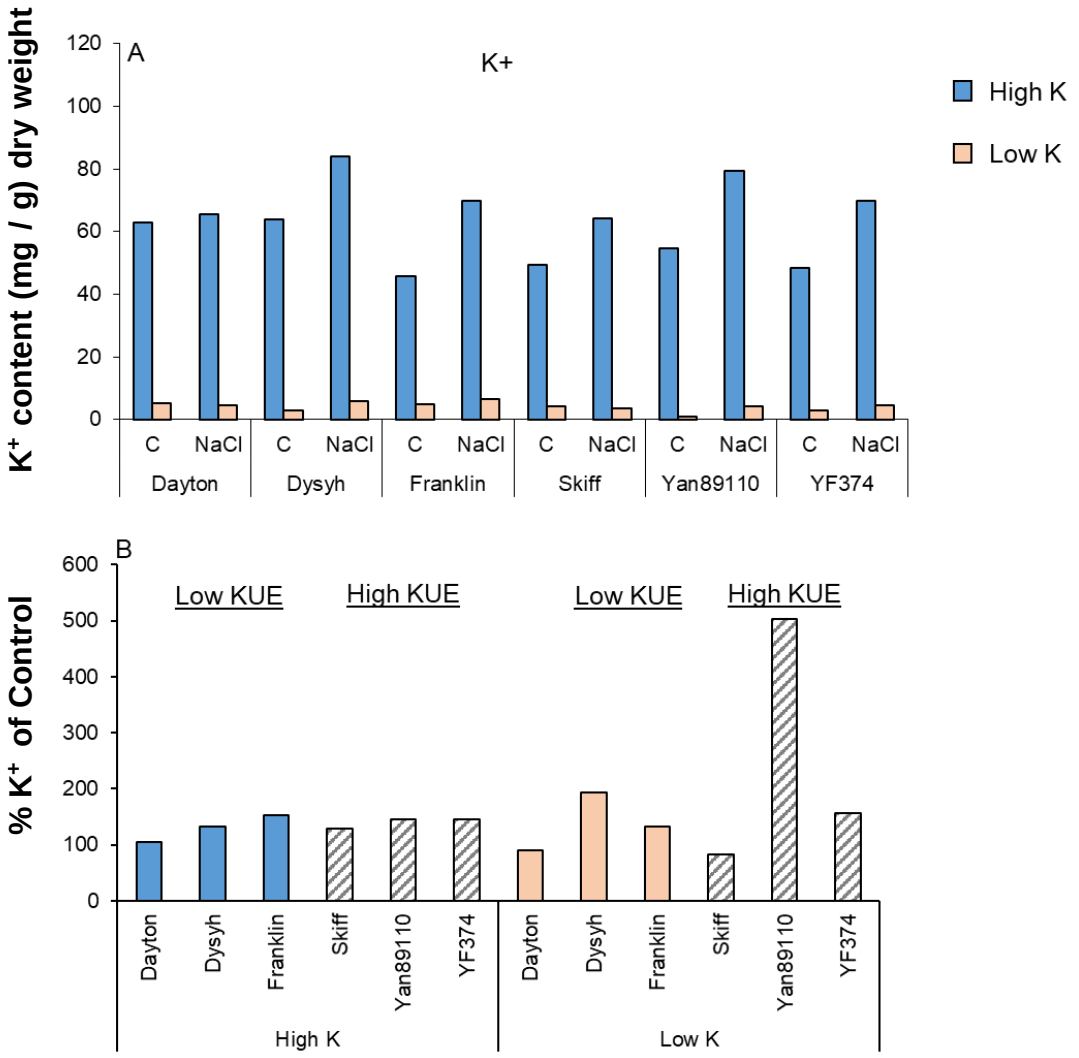

Figure 5.9 (A) Effects of salinity and K^+ treatment on K^+ in K-efficient (Skiff, Yan 89110 and YF374) and K-inefficient (Dayton, DYSYH and Franklin) genotypes of barley. Seedlings were subjected to one of two treatments of K^+ (low: 0.002 mM; high: 20 mM); in either absence (Control) or presence of salinity stress imposed by irrigating with 300mM NaCl. (B) Relative concentration of K^+ in barley genotypes grown under salinity (as described for Figure 5.12A), showing concentration as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

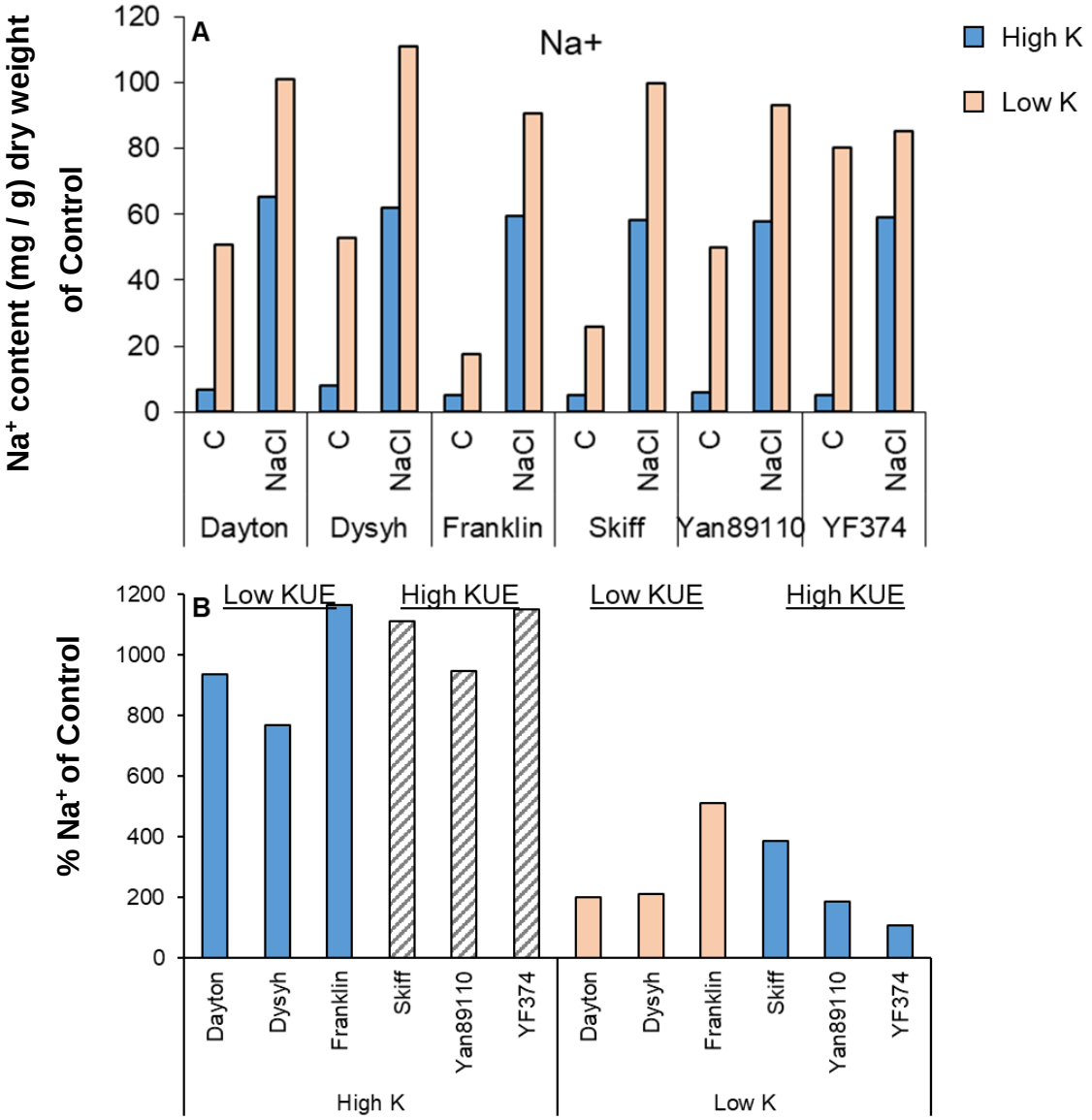

Figure 5.10 (A) Effects of salinity and K^+ treatment on Na^+ in K-efficient (Skiff, Yan 89110 and YF374) and K-inefficient (Dayton, DYSYH and Franklin) genotypes of barley. Seedlings were subjected to one of two treatments of K^+ (low: 0.002 mM; high: 20 mM); in either absence (Control) or presence of salinity stress imposed by irrigating with 300mM NaCl. (B) Relative concentration of Na^+ in barley genotypes grown under salinity (as described for Figure 5.13A), showing concentration as a percentage of the value under identical non-saline conditions. High KUE genotypes are grouped for contrast with low KUE genotypes.

5.4 Discussion

Salt stress generally restricts the crop growth rate and produces smaller leaves, shorter stature and reduced economic yield (Hasanuzzaman et al. 2013; Shannon & Noble 1990). The current study revealed the effect of salinity stress on growth, yield, physiological attributes and K^+ use efficiency (KUE) of six barley genotypes. Exogenous application of K^+ used to ameliorate the toxicity of salt stress was shown to improve the photosynthetic activity, crop growth, KUE, and physiology of barley.

Barley is considered to be a relatively salt tolerant crop. However, in the current study, salt stress had a severe effect on the biomass of barley genotypes. The reduction in the biomass under salinity stress may be due to a reduction in the photosynthetically active leaf area by the chlorosis and necrosis of leaves (De-Herralde et al. 1998; Ivanov 2015). The reduction in biomass might also be due to the development of soil moisture stress under salt stress, which suppresses plant growth (Mane et al. 2011; Ashraf et al. 2011; Kausar & Gull 2014). Na^+ accumulation in plant cells damages plant metabolism and results in growth reduction. Mano et al. (1996) evaluated 6172 barley genotypes having diverse origins, and 368 genotypes from isogenic lines from China, Korea, Turkey and Japan under salinity stress conditions. They reported in terms of biomass production the genotypes from China and Korea were more tolerant as compared to those from Turkey and Japan. The current study demonstrated salinity stress tolerance in the tested genotypes ranged from highly sensitive to very tolerant.

Yield components in terms of tiller number, spike number, grain weight and grain yield of barley genotypes were determined under salt stress. Results revealed drastically reduced yield components. A reduction in the grain filling was observed, indicating lower set and higher sterility in salinised plants. The reduction in the seed set could be due to the failure of stigma receptivity. Under salinity stress most genotypes didn't produce any grain, regardless of K^+ treatment, although some K-efficient genotypes produced small amounts of grain that could be weighed. Salt stress delays and reduces crop flowering and yield through affecting pollination and seed set and formation (Maas et al. 1996; Sharbatkhari et al. 2016). A reduction of yield components under salinity stress may also be attributed to the production of foliage having impaired expansion, early senescence, thus reducing the photosynthetic rate (Park et al. 2015; Rasul et al. 1997). Salt stress

shrivels the seeds and lowered the yield. The reduced yield under salinity stress might also be due to a reduction in the daytime efficiency of plants to develop and fill seeds.

In the present study, salinity stress caused a drastic reduction in stomatal conductance of the tested genotypes. Traditionally, this reduction is attributed to be a result of the osmotic component of the salt stress. Indeed, salt stress disturbs the water balance in plants due to high solute concentration in the root zone, impacting the opening and closing of stomata, which would cause reduction in photosynthesis and stomatal conductance (Dubey 2005). In this context, application of K^+ in saline conditions was expected to ameliorate salt stress and promote stomatal conductance. However, this was not the case (Figure 5.7). With one exception (variety DYSYH; treated as outlier) the impact of salinity on Gs was independent of K^+ availability in the soil, ruling out direct osmotic effect. At the same time, the clear difference in grain yield responses and overall stronger performance of high K^+-efficient cultivars under saline conditions (Figure 5.1, Figure 5.2) suggest a causal link between K^+ availability and a plant's adaptive responses to salinity.

Salinity stress causes ion toxicity that limits plant growth. The cytosol in plant cells hardly tolerates Na^+ above 20 mM (Amtmann & Sanders 1999; Blumwald & Aharon 2000; Walker et al. 1996). While some plants appear to utilise Na^+ to a certain concentration, it will be problematic if the concentration of Na^+ becomes high (Kronzucker et al. 2013). The similarity between Na^+ and K^+ is beneficial for many halophytic plants to grow in environments of high salt concentration, since they reduce the K^+ concentration required for basic metabolic activity. Many non-halophytic plants under limited K^+ supply can utilise Na^+ together with Mg^{2+} and Ca^{2+} to replace K^+ in the vacuole as an alternative (Flowers & Läuchli 1983; Kronzucker & Britto 2011). Reduced K^+ content in the leaves has been associated with the presence of tissue Na^+ because K^+ can be replaced by Na^+ in many functions (Besford 1978; Subbarao & Johansen 2002).

Increases in grain yield were accompanied by increases in grain number, which therefore might be a driver for the increase in yield. Surprisingly, two of the K-inefficient genotypes yielded more under low K^+ treatment (in the absence of salinity) than they did with high K^+ treatment. The increased yield can be postulated to have been the result of a change in resource partitioning that was induced by K^+ deficiency, or the result of a stress induced cross tolerance that improved a normally limiting trait, such as pollen viability. In Chapter 4 (Table 4.5) it was found that Dayton

and DYSYH were amongst the highest varieties for leaf sap osmolality under low K^+ treatment, but amongst the lowest for osmolality under high K^+ treatment. In the absence of K^+ as an osmolyte, the high osmolality is likely to have been due to the use of high concentrations of organic solutes. The devotion of resources to the production of these organic osmolytes, which being mobile would then have been available for grain production, may have improved the partitioning of resources towards grain. High concentrations of organic solutes would also have produced wide ranging cross tolerance that might have improved the performance of yield limiting traits (Puniran-Hartley et al 2014).

The reduced chlorophyll concentrations observed in the current study under salinity stress may be due to an accumulation of toxic ions under salinity stress (Nawaz et al. 2010; Delfine et al. 1999; El-Hendawy et al. 2005; Dulai et al. 2014), or be associated with increased production of ROS (Bose 2014). Surprisingly, the relative increase in the shoot Na^+ content was much stronger under high K^+ supply (Figure 5.10) ruling out a direct effect of the Na^+ toxicity. Hence, it is plausible to suggest that the reported difference in salinity stress responses between low- and high-KUE genotypes may be attributed to differential ROS production and scavenging between these two groups.

The relationship between the concentration of various ions in both the cell cytosol and apoplast and the amount of ROS produced is complex. On the one hand, both mono- and divalent cations induce $^{\bullet}O_2^-$ production through the activity of NADPH oxidase (Kawano et al 2001). At the same time, activity of many antioxidant enzymes (e.g. CAT or SOD) is critically dependent on availability of some nutrients (Yang and Poovaiah 2002). Also, K^+ deficiency increases NADPH-dependent $^{\bullet}O_2^-$ generation in root cells (Cakmak 2005), and the suppression of an NADPH oxidase in Arabidopsis *rhd2* mutant prevented the up-regulation of genes that are normally induced by K^+ deficiency (Shin & Shachtman 2004).

It is often said that high KUE is likely to involve the substitution of Na^+ for K^+. In the current work it was seen that the K-efficient genotype YF374 underwent a dramatic increase in Na^+ concentration in response to a low K^+ treatment, even in the absence of salinity treatment. While all genotypes, both K-efficient and K-inefficient, did increase considerably in Na^+ content in response to K^+ deficiency, the increase was far stronger in YF374 than in any other, and indicates

that in this genotype Na^+ use is an important aspect of KUE. Other K-efficient genotypes didn't necessarily have a stronger response of Na^+ to low K^+ than did the K-inefficient types. YF374 has a high concentration of organic solutes, (Chapter 4, section 4.3.8), which may be one of the features that allows it to use Na^+ as an osmolyte without damage.

Under non-stressed conditions, (high K^+ and non-saline), a positive relationship existed between Na^+ and K^+, such that genotypes with high whole shoot K^+ concentrations also had high concentrations of Na^+ relative to other genotypes, and those with relatively low K^+ also had low Na^+. This suggests that under non-stress conditions the concentrations of these two ions are similarly governed by the requirement for inorganic osmolytes, which will vary according to genotypic differences in strategies for maintaining osmotic homeostasis (Zarei et al 2018). This positive relationship between Na^+ and K^+ was not present under stress (salinity or K^+ deficiency), because under stressful conditions the relationship between K^+ and Na^+ becomes governed by differing abilities of genotypes to substitute Na^+ for K^+, and to tolerate or exclude Na^+ at potentially toxic concentrations.

The fact that all three K-efficient genotypes outperformed all three K-inefficient genotypes when under salinity is a strong indication that tolerance towards salinity can be used when selecting for K-efficiency, and vice versa. There are several overlapping traits in the physiology of salt tolerance and K-efficiency which are likely explanations; including ability to produce high concentrations of organic solutes to either balance excess Na^+ or compensate for a deficiency of K^+, the ability to use Na^+ as an osmolyte (which is in part again dependent on ability to produce compatible solutes), and an ability to selectively take up and translocate potassium to maintain a proper K/Na ratio. The fact there are so many possible traits with overlapping benefits for salinity tolerance and K-efficiency means that there are likely to be multiple aspects of tolerance to salinity and K^+ deficiency that could be used for selecting genotypes with likely cross tolerance. In the current study there is a possible indication of this in the fact that Yan 89110, which had very high leaf K^+ but not especially high yield under K^+ deficiency, proved to be relatively tolerant of salinity along with the genotypes that had been selected on the basis of higher yield under low K^+ treatment. However the salinity tolerance of Yan 89110 was not as strong as that of the other two K-efficient genotypes, so high leaf K^+ might not be as good an indicator of salinity tolerance as

yield under K^+ deficiency is. A strong statement about this can't be made on the basis of only three genotypes.

Ability to maintain a high K/Na ratio has previously been suggested to be an important aspect of salinity tolerance, and a practical selection target when selecting for salinity tolerance (Shabala 2013; Shabala and Pottosin 2014). From the current study it appears that K-efficiency and salinity tolerance can be combined, and that selecting for one is likely to improve the other, which makes simultaneous improvement in K-efficiency and salinity tolerance relatively easy. This has practical significance. Amongst the practical implications is that the large amount of research effort being spent on salinity tolerance is likely to produce incidental benefits in K-efficiency, with subsequent reduced spending on K^+ fertilisation. Considering that it has also been shown that barley genotypes vary greatly in their optimum level of potassium fertilisation, with efficient genotypes (that are likely to be salt tolerant) tending to reach peak yield with lower K^+ rates, (Chapter 3), it may be a worthwhile investment to test new salt tolerant barley varieties to determine if they warrant a reduced recommendation for K^+ fertiliser.

Throughout this study, the genotype YF374 has stood out for its combination of K-efficiency traits (notably high leaf K^+ and high yield under K^+ deficiency), along with high yield potential when supplied with sufficient K^+. In the current chapter, YF374 was also found to perform comparatively well under salinity. This interesting genotype appears to be a good candidate for a breeding programme to select simultaneously for K-efficiency and salinity tolerance with high yield potential.

Chapter 6 General Discussion

Adequate concentrations of K^+ is needed for optimal protein synthesis, photosynthesis and enzyme activation (Szczerba et al. 2009). Developing barley cultivars with a high impact on K^+ use efficiency under deficient conditions can be an effective approach to overcome K^+ deficiency.

Salt stress generally restricts the crops growth rate and produces smaller leaves, shorter stature and reduced economic yield (Hasanuzzaman et al. 2013; Shannon & Noble 1990). But the application of K^+ alleviate the antagonistic effect of salt stress. So, the morphological and physiological response of barley to K^+ availability and the effect of salinity on these responses have been studied in two separate experiments.

Barley response to K^+ supply in the present study varied with K^+ levels: the availability of K^+ in the soil had a major effect on yield components, tiller number, plant height, leaf sap osmolality, chlorophyll florescence, chlorophyll contents and leaf and xylem K^+ content. The improvement in the studied traits is likely to be attributable to the activation of enzymes, protein synthesis and increased uptake of other nutrients that was reflected by plant growth.

An increase in grain yield in the response to K^+ application was correlated with an increase in numbers of spikes and grain numbers, but tiller number did not correlate with grain yield. It is likely that this is the result of reduced fertility of tillers due to resource limitations. Also, the grain yield showed no direct correlation with leaf K^+ content, at either low (0.002 mM) or high (20 mM) K^+ treatments. However, a significant positive correlation was observed between grain yield and xylem K^+ concentration under severe K^+ deficiency conditions. This suggests that the ability to control xylem K^+ loading and re-translocate K^+ to developing sinks is more essential for KUE in barley, as compared with the ability of roots to acquire K^+.

Our findings reported here suggest that K^+ availability might contribute to dropping the gap between the potential yields and realized a yield of barley. According to Sweeney et al. (2000) & Sharma et al. (2005), an increase in grain yield with supplementation of K^+ could be due to an improvement in the kernel weight.

Properly managed K^+ fertility in soil is essential for maximum grain yield. Barley genotypes showed variable response with the application of different K^+ levels. The highest efficient raise in plant biomass and yield components was attributed to the K^+ application up to 0.02 mM and it could be the threshold level to optimize yield production. The availability of K^+ below the threshold level of K^+ can be considered deficient for barley. Dry biomass and grain yield attributes were decreased with 0.002 mM K^+ supplementation. There are economic losses in the form of reduced biomass and grains production due to a reduced K^+ availability. This reduction in yield could be attributed to the reduced production of photosynthetic assimilates with insufficient K^+. Although, the lowest K^+ availability (0.002 mM) does not support optimum yields but may prevent critical deficiencies

The results of the current study suggested a competitive interaction between K^+ and Na^+ and their transport into plant parts. Similar results were reported by (Rodrigues et al. 2012) where, under a similar K^+ concentration (10 mM) in the rhizosphere, plants showed a higher selectivity of K^+ over Na^+ and higher rates of loading K^+ into the xylem compared with Na^+.

The osmolality of barley plants showed an increasing trend towards the lowest performing group under limited K^+ supply conditions. In the current experiment, a broad range of leaf sap osmolality was observed within each K^+ treatment. These results are similar to those of Boscari et al. (2009), who studied K^+ channels during barley leaf growth and development. Leaf sap osmolality drives the cell expansion through continuous uptake of water (Fricke 2002). K^+ is the main osmoticum in leaf epidermal cells of grasses contributing 50% of osmotic pressure (Fricke et al. 1994; Wu et al. 2015).

Genotypic differences in K^+ use efficiency also influenced K^+ uptake and stress tolerance: K^+ efficient cultivars were more tolerant of low K^+ availability than K^+ inefficient cultivars. The difference between the K^+ efficient and the inefficient genotypes involved not only the uptake of K^+ but also its transport and use (Wang et al. 2007). The mechanisms for genotypic variation in K^+ efficiency may be due to the effective uptake from soil and/or efficient utilization of K^+ (Sattelmacher et al. 1994).

The results of the present study revealed the genetic potential of barley genotypes to perform under minimal K^+ availability. At the lowest K^+ supply of 0.002 mM, the best performing genotypes were Gebeina, Skiff, YF374 and Flagship. The less effective genotypes were Dayton, DYSYH,

and RGZLL (Table 3.5-3.7). These genotypes may therefore be recommended for mapping DH populations, to reveal QTLs responsible for KUE in barley.

In terms of K^+ utilization efficiency, cultivars may differ in their ability to translocate K^+ from roots to other plant portions (Dong et al. 2015; Sattelmacher et al. 1994). K^+ utilization efficiency can be calculated by taking into account both yield and K^+ concentration in the plant (Gourley et al. 1994; Khoshgoftarmanesh et al. 2010). Several studies have associated potassium utilisation efficiency with genotypic differences in K^+ concentrations in shoots for barley (Sharma et al. 2004; Wu et al. 2011), canola (Damon et al. 2007; George et al.2002; Tian et al. 2008), and sweet potato (George et al.2002). In Australia, two thirds of cultivated land are K^+ deficient (Rengel & Damon 2008), and K^+ fertilizers are important inputs, which increase the cost of production for farmers. The present study suggests that cultivars Yan89110, TF026, Yan90260, Yu6472, Tx9425, YSM3 and YF374 may be useful to breeding programmes to improve K^+ efficiency.

Barley is considered as a salinity tolerant crop however in the current study, salt stress had a deleterious effect on the fresh and dry biomass of barely genotypes. The result revealed that the exogenous application of K^+ ameliorated the toxicity of salt stress and improved the photosynthetic activity, crop growth, KUE, and the physiology of barley. The reduction in biomass under salinity stress may be due to a reduction in photosynthetically active area that is caused by the chlorosis and necrosis of leaves (De Herralde et al. 1998; Ivanov 2015). A reduction in fresh weight might also be due to the development of soil moisture stress under salt stress which suppresses plant growth (Mane et al. 2011). The application of K^+ alleviated the antagonistic effect of salt stress and was effective in promoting fresh and dry weights and the biomass of barley genotypes. The increase in the K^+ supply led to an increase in fresh weight in all varieties, although the extent of responses differed significantly between genotypes. The effectiveness of the K^+ application under salinity stress is well-known in many crops (Ashraf et al. 2012; Ashraf et al. 2015; Hussain et al. 2013), possibly due to the function of K^+ in the activation of enzymes necessary for plant growth.

The application of K^+ in saline conditions relieved symptoms of salt stress and promoted stomatal conductance. Stomatal conductance due to K^+ application under salt stress might be due to the role of K^+ as an osmoticum that maintains the moisture content in plant tissues (Marschner 1995).

Salt tolerant genotypes showed better chlorophyll content and fluorescence as compared to sensitive genotypes. The reduced chlorophyll contents under salinity stress may be due to an accumulation of toxic ions, which disorders the opening and closing of stomata, thus causing rapid maturing of leaves under salinity stress (Nawaz et al. 2010).

The current study demonstrated the application of K^+ significantly ameliorates the toxicity of Na^+ and promotes plant growth. Antagonistic effects of K^+ and Na^+ have also been reported by (Lynch & Läuchli 1984). Bohra & Doerffling (1993) have likewise reported the improvement of growth and dry matter production in rice (*Oryza sativa* L.) under saline conditions with the applications of K^+. The higher K^+ uptake of salt tolerant genotypes may be due to their capacity to select K^+ over Na^+. Carden et al. (2003) & Abbasi et al. (2014) also reported the higher K^+ accumulation in barley and maize plants due to their preferential loading of K^+ rather than Na^+ into xylem contents. Salt tolerant barley genotypes possess a strong affinity for K^+ over Na^+ and thus achieve a more favourable K^+/Na^+ ratio as compared to sensitive barley genotypes. Similar reports for cucumber (Kaya et al. 2001) and olive (Chartzoulakis et al. 2006) have shown that the application of K^+ ameliorates the toxicity of Na^+ and increases the K^+/Na^+ ratio (Carden et al. 2003). Previously, Akram et al. (2012), Abbasi et al. (2014), Akram et al. (2012) and Lynch & Läuchli (1984) also reported higher K^+/Na^+ ratios in salt tolerant genotypes.

Conclusion

This study reported the potential of K^+ efficient genotypes to identify markers for mechanisms that causes efficient K^+ consumption. It can be suggested that the genotypes Gebeina, Skiff, YF374, and Flagship could be used in breeding programs to improve K^+ efficiency regarding grain yield. So far, progress towards developing K^+-efficient cultivars suitable for integration into production systems has been slow, and the selection for K^+ utilization efficiency may be effective across field environments (Smith et al. 1999). A better understanding of the relevant efficiency mechanisms and their associated markers will benefit breeding programs and enable marker-assisted selection of effective genotypes.

Chapter 7 References

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